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The Effect of Yeast Feeding upon Experimentally Produced Liver Cancer and Cirrhosis

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o-Aminoazotoluene (21) and *p*-dimethylaminoazobenzene (6) are equally effective when given orally in producing liver cancer in rats. This change occurs after 300 days when *o*-aminoazotoluene is used and after only 150 days with *p*-dimethylaminoazobenzene. In some instances, however, liver cancers are produced by either substance at earlier time intervals. The sequence of events is not the same with both dyes. When *p*-dimethylaminoazobenzene is used, the usual sequence is first the formation of cirrhosis which is generally manifest at the end of 60 days. As a rule, by 90 days most of the livers show cholangiomas and at this time some hepatomas are also present. Then, as time goes on, the production of hepatomas increases. Ordinarily by the 150th day practically all the livers are the seat of a cancerous process (both cholangiomas and hepatomas). With *o*-aminoazotoluene the first pathological alteration is usually a hepatoma formation or occasionally the development of cholangioma. Cirrhosis generally does not develop and if it does is usually not pronounced. The malignant transformation of the liver cells to hepatomas occurs more slowly with *o*-aminoazotoluene than with *p*-dimethylaminoazobenzene and takes place in about 300 days.

Dietary factors greatly influence the formation of liver cancer by either of the above-mentioned chemicals. Experiments have shown that the production of liver cancer by *o*-aminoazotoluene or *p*-dimethylaminoazobenzene is definitely reduced when wheat (1, 2, 3, 8, 19, 24), rye (10), or millet (15) has been used as a basal diet instead of rice.

It is also known that the addition of yeast (7, 12, 16, 23); liver (12, 14, 17, 18); kidney (13); rice-bran oil (9, 20); ether extracts of yeast (23); ether extracts of rice-bran (23); the water-soluble, alcohol-insoluble portion of whole liver (12); or the water-soluble, alcohol-soluble portion of whole liver [1] (22) to a rice diet increased resistance to tumor development.

The failure of *p*-dimethylaminoazobenzene to produce liver cancer in rats maintained on the rice diet supplemented with riboflavin and casein, or with a combination of thiamine, riboflavin, pyridoxin, pantothenic acid, cystine, and choline has also been reported (4, 5, 12).

The object of the present study is to find whether or not primary tumors of the liver, hepatoma and cholangioma, can be treated successfully with the anticarcinogenic substances mentioned above. In these experiments albino rats were maintained on a basal diet of rice, and brewer's yeast was added after the formation of the neoplasms, since it has been demonstrated to have a definite protective action in preventing the development of liver cancer in animals fed *p*-dimethylaminoazobenzene or *o*-aminoazotoluene.

EXPERIMENTAL

Liver tumors were induced in young adult male albino rats of the Sherman stock by feeding butter yellow [2] (*p*-dimethylaminoazobenzene, No. 338, Eastman Kodak Company). The dye was dissolved in olive oil to form a 3 per cent solution. Twenty cc. of this solution were evenly mixed with 1,000 gm. of coarsely ground, unpolished rice (brown rice). The rats were allowed to take as much of the mixture as they desired. The basal diet was supplemented with a small amount of fresh carrot daily (about 1 gm. per rat).

The results obtained from the continuous feeding on a butter yellow-rice diet may be summarized briefly as follows (23).

During the first 30 days' feeding on butter yellow,

[1] Liver extract, No. 343, Eli Lilly Company.

[2] In the course of the investigation a study was made on the degree of carcinogenicity of purified butter yellow. This was kindly prepared by Mr. W. C. Bainbridge of H. Kohnstamm Company, New York, from *p*-dimethylaminoazobenzene, No. 338, Eastman Kodak Company, by twice recrystallizing from isopropanol. It possessed a melting point of 118° C. The results of the comparative experiments showed that the incidence of liver cirrhosis and cancer in rats fed the purified butter yellow was the same as in those fed original butter yellow.

the livers of rats showed no great changes in the gross. Microscopic sections of the liver revealed no evidence of cirrhosis or transformation of liver cells into bile duct carcinoma (cholangioma) or hepatoma (Fig.1 A).

At the end of 60 days most of the livers revealed the irregular surfaces with more or less well-defined nodules (Fig. 1 B,C,D,E). Histologically there was extensive proliferation of the fibrous connective tissue with lymphocytic infiltration, with isolated islands of

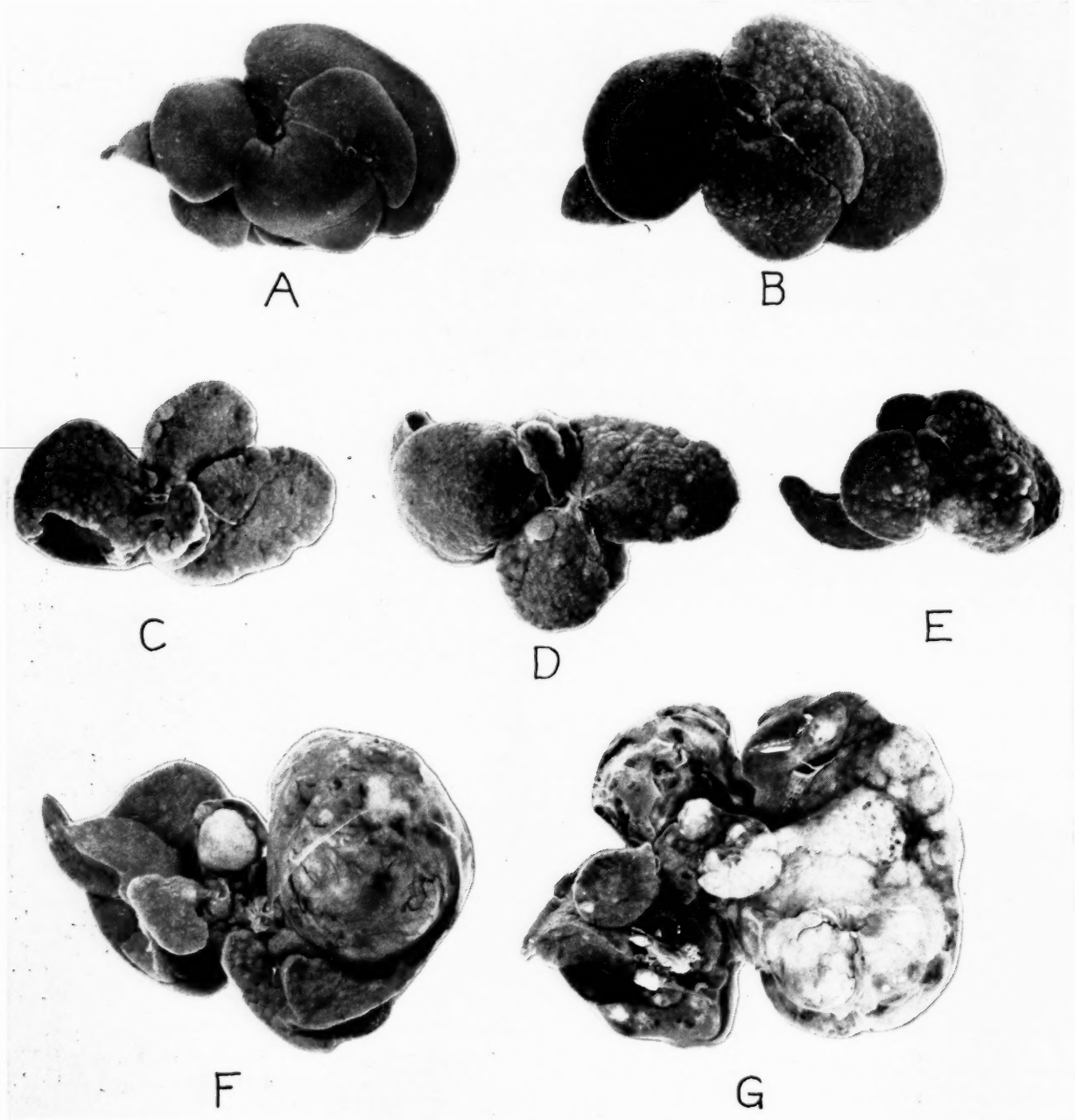

FIG. 1.—Gross appearance of livers of rats fed on butter yellow-rice diet for various lengths of time. Photographs of the livers were made at the same magnification.

A. Liver of a rat (30 days). Practically normal gross appearance.

B,C,D,E. Livers of rats (60 days). Early cirrhosis to nodular cirrhosis.

F,G. Livers of rats (120 days). Hepatomas, either isolated or very numerous.

At the end of 45 days of butter yellow feeding, some livers (less than 5 per cent) had irregular surfaces with more or less well-defined nodules, which revealed early cirrhosis and adenomatous hyperplasia of bile ducts.

hyperplastic liver cells persisting and presenting the typical picture of cirrhosis. Some nodules (about 20 per cent) at this stage already showed adenomatous hyperplasia of the bile ducts with beginning malignant transformation into a cholangioma or hepatoma.

At the end of 85 days of butter yellow feeding, the livers contained many large and small nodules. Microscopic sections of these nodules demonstrated typical cholangiomas, hepatomas, or both (about 80 per cent).

At the end of 120 days of butter yellow feeding, practically all livers exhibited large, isolated, massive nodules, microscopic sections of which showed hepatomas, cholangiomas, or both in the same liver (Fig. 1 F,G). Occasionally metastases were found in the mesentery, omentum, and lung.

Effect of Withdrawing Butter Yellow from the Basal Rice Diet

The foregoing paragraphs present the various liver changes resulting from butter yellow feeding to the development of advanced hepatic cancer.

The following paragraphs describe the effect of the removal of butter yellow from the diet, after a definite period of ingestion, upon the incidence of liver cancer.

As in previous experiments, young adult male rats of the Sherman stock were maintained on a butter yellow-rice diet for definite periods of time. Then the carcinogen was removed from the food and feeding was continued until the animals either succumbed or were sacrificed.

The results obtained from this study are summarized in Tables I and III.

It is evident that no cirrhosis or cancer developed in the livers of any rats if butter yellow had been given for less than 32 days, and in very few even after the preliminary feeding with butter yellow for 45 days. However, if the preliminary feeding with butter yellow exceeded 60 days, liver cancer developed subsequently in the animals surviving on a basal rice diet. In longer periods on a butter yellow-rice diet, a greater incidence of liver cancer resulted. This agrees with the observation of Kinosita (6). Rats Nos. 24, 35, 46, 51, and 53 had metastases in the mesentery; rat No. 52, in the mesentery, omentum, and peritoneum; rat No. 45, in the mesentery, omentum, and lung; and rat No. 44, in the lung.

Effect of Adding Whole Yeast to the Basal Rice Diet Following Periods upon a Butter Yellow-Rice Diet

In the above experiments, rats fed the butter yellow-rice diet for 60 days had cirrhotic livers in the majority of cases. At this time, 20 per cent of the livers also showed adenomatous hyperplasia of the bile ducts with beginning malignant transformation into a cholangioma or hepatoma. It seems that the origin of cancer, or a certain change which leads to the cancerous anaplasia, can occur in a short period (60 days), if a relatively large amount of butter yellow is administered (each animal consumed about 186 mgm. of butter yellow for the first 60 days). The withdrawal of butter yellow from the diet at this stage did not lessen the subsequent development of liver cancers.

It is evident that once a precancerous condition has been established in the liver it is difficult to re-establish a normal physiological state by means of a rice-carrot diet.

Rice is extremely poor in vitamins and protein. The rats on the rice diet were able to maintain their weights in some instances, others declined slowly, and only a few gained slightly.

It is now known that the protective supplements against hepatoma formation are generally rich in both protein and the vitamins of the B complex, especially riboflavin (4, 5, 12).

The succeeding paragraphs deal with attempts to ascertain whether a destructive action against experimental liver tumors can be accomplished by the addition of yeast (rich in protein and vitamin B complex) to the basal diet after the formation of hepatic cancers, or precancerous states, resulting from the previous ingestion of butter yellow.

Following the procedure described above, we maintained rats on a butter yellow-rice diet for definite periods of time. Then the carcinogen was removed from the food and feeding was continued on the basal unpolished rice diet (85 per cent) with a 15 per cent supplement of whole yeast to the diet, until the animals either succumbed or were sacrificed.

The results are presented in Tables II and IV.

The data in Tables II and IV show clearly that yeast feeding produced a striking inhibition of liver cancer development. In 16 rats which received the preliminary feeding with butter yellow for 62 to 63 days, followed by rice-yeast diet without butter yellow for 100 to 240 days, 12, or 75 per cent, had smooth, practically normal livers. In contrast, of animals fed the butter yellow-rice diet for the same period, followed by rice alone, only 11 per cent had histologically normal livers.

Cirrhosis or cancer was absent in the livers of rats if butter yellow had been given for less than 45 days. However, if the preliminary feeding with butter yellow exceeded 85 days, liver cancer resulted in 100 per cent of the cases, including the production of disseminated metastases in the mesentery and omentum (rats Nos. 43, 45, 46, 49, 52), even though the dietary anticarcinogen (yeast) was fed for long periods of time subsequent to the butter yellow ingestion.

Aspiration biopsies were performed on the livers in 25 cases to establish the presence of liver tumors (11). About 75 per cent of the livers of rats maintained

TABLE I: CHANGES IN THE LIVERS OF RATS FED ON A BUTTER YELLOW-RICE DIET FOR 32 TO 131 DAYS, THEN ON RICE ALONE

Rat number	Number of days fed butter yellow and rice	Body weight		Number of days fed rice alone	Final body weight, gm.	Liver findings *
		Initial, gm.	Final, gm.			
1	32	134	145	34 †	95	—
2	32	123	115	114 †	120	—
3	32	125	92	123 †	115	—
4	32	125	128	126 †	134	—
5	32	123	134	160	167	—
6	32	130	126	160	150	—
7	32	130	143	160	175	—
8	32	124	130	160	170	—
9	32	140	145	160	180	—
10	32	130	150	160	183	—
11	32	137	119	160	154	—
12	45	113	118	79 †	87	—
13	45	137	159	90 †	150	—
14	45	124	125	120	134	—
15	45	123	108	120	127	—
16	45	129	136	151	142	—
17	45	111	149	151	100	—
18	45	115	100	116 †	125	+
19	61	122	124	150	135	—
20	61	127	98	100	90	±
21	61	138	150	98 †	137	+
22	61	130	100	120	102	+
23	61	129	145	176 †	110	+
24	61	124	127	112 †	140	++
25	61	130	131	112 †	139	++
26	61	129	147	124 †	152	++
27	61	139	130	166 †	117	++
28	71	146	145	85	125	±
29	71	128	108	150 †	103	±
30	71	120	89	28 †	90	+
31	71	131	145	69	100	+
32	71	127	91	80 †	92	+
33	71	149	154	100	120	+
34	71	134	85	108 †	90	+
35	71	133	140	169 †	109	++
36	81	194	170	55 †	115	±
37	81	222	156	70	161	+
38	81	213	201	94	177	+
39	81	171	120	79	136	++
40	81	224	167	83	146	++
41	84	156	147	304	145	±
42	84	181	200	54 †	175	+
43	84	162	93	54	72	+
44	84	145	112	150 †	100	++
45	84	181	165	159 †	112	++
46	84	178	165	167 †	104	++
47	131	130	162	25 †	181	+
48	131	180	136	57 †	130	+
49	131	154	123	58 †	140	+
50	131	125	140	48 †	131	++
51	131	92	103	50 †	108	++
52	131	167	161	56 †	165	++
53	131	135	148	60 †	160	++
54	131	148	136	72 †	146	++

* — indicates smooth, practically normal liver; ±, nodular cirrhosis with adenomatous hyperplasia; +, distinct areas of cholangioma or hepatoma, or both; ++, extensive liver cancer with or without metastasis. In all cases the diagnoses were confirmed by microscopic examination.

† Died.

TABLE II: CHANGES IN THE LIVERS OF RATS FED ON A BUTTER YELLOW-RICE DIET FOR 32 TO 131 DAYS, THEN ON A WHOLE YEAST-RICE DIET

Rat number	Number of days fed butter yellow and rice	Body weight		Number of days fed rice and yeast	Final body weight, gm.	Liver findings *
		Initial, gm.	Final, gm.			
1	32	140	144	22 †	140	—
2	32	120	125	95 †	147	—
3	32	116	121	100	147	—
4	32	134	133	100	174	—
5	32	121	106	120	150	—
6	32	122	115	121	129	—
7	32	125	110	145	155	—
8	32	120	125	155	159	—
9	32	131	126	155	170	—
10	32	115	124	175	158	—
11	45	124	120	103	160	—
12	45	114	106	103	139	—
13	45	116	119	132	145	—
14	45	120	117	132	120	—
15	45	126	122	132	154	—
16	45	99	102	170	142	—
17	45	98	97	170	145	—
18	55	112	104	77	134	—
19	55	115	70	239	105	—
20	55	140	130	240	178	—
21	55	112	104	77	127	±
22	55	111	118	77	132	+
23	55	115	100	102 †	111	+
24	62	147	182	67	191	—
25	62	142	120	112 †	159	—
26	62	145	161	112 †	175	—
27	62	142	144	112	188	—
28	62	135	154	165	175	—
29	62	133	171	165	179	—
30	62	149	193	165	210	—
31	62	144	156	165	150	—
32	62	125	174	165	179	—
33	62	130	81	25 †	120	±
34	63	121	115	100	163	—
35	63	120	152	150	165	—
36	63	122	120	150	152	—
37	63	118	107	185 †	105	±
38	63	120	129	112 †	100	+
39	63	120	136	151 †	100	+
40	63	123	165	240 †	145	+
41	85	134	86	37 †	95	+
42	85	123	100	74 †	125	+
43	85	154	133	91 †	136	++
44	85	152	115	105 †	185	++
45	85	127	110	115 †	143	++
46	85	135	143	159 †	120	++
47	131	144	116	132	133	+
48	131	136	100	50 †	125	++
49	131	185	160	74 †	170	++
50	131	145	150	85 †	131	++
51	131	140	104	93 †	141	++
52	131	165	95	132	184	++

* See Table I for explanation of symbols.
† Died.

TABLE III: RESULTS OF BASAL RICE DIET FOLLOWING BUTTER YELLOW-RICE DIET ON THE PRODUCTION OF LIVER CANCER

Number of animals used	Number of days fed butter yellow and rice	Number of days fed basal rice diet subsequent to butter yellow-rice diet	Liver changes *				Incidence of liver cancer, per cent
			−	±	+	++	
10	32	114–160	10	0	0	0	0
7	45	79–151	6	0	1	0	14
9	61	98–176	1	1	3	4	78
7	71	69–169	0	2	4	1	71
5	81	55–94	0	1	2	2	80
6	84	54–304	0	1	2	3	83
7	131	48–72	0	0	2	5	100

* See Table I for explanation of symbols.

TABLE IV: RESULTS OF ADDING WHOLE YEAST TO BASAL RICE DIET FOLLOWING PERIODS ON BUTTER YELLOW-RICE DIET, ON THE PRODUCTION OF LIVER CANCER

Number of animals used	Number of days fed on butter yellow and rice	Number of days fed rice and yeast diet subsequent to butter yellow-rice diet	Liver changes *				Incidence of liver cancer, per cent
			−	±	+	++	
9	32	95–175	9	0	0	0	0
7	45	103–170	7	0	0	0	0
6	55	77–240	3	1	2	0	33
9	62	112–165	9	0	0	0	0
7	63	100–240	3	1	3	0	43
5	85	74–159	0	0	1	4	100
6	131	50–132	0	0	1	5	100

* See Table I for explanation of symbols.

on butter yellow-rice diet for 85 days or longer gave positive tests.

Table II demonstrates that 83 per cent of the animals fed the yeast and rice diet following the butter yellow-rice diet gained weight and further presented a healthy appearance throughout the experimental period, even though cancer was present in the livers.

SUMMARY

1. The therapeutic action of yeast upon liver cirrhosis and cancer has been investigated. The liver changes were induced in rats by feeding unpolished rice and *p*-dimethylaminoazobenzene.

2. Cirrhosis was absent in the livers of rats if butter yellow had been given for less than 32 days. However, if the preliminary feeding with butter yellow exceeded 60 days, liver cancers developed in a large percentage of animals surviving on a rice diet fed subsequent to the butter yellow-rice diet for over 100 days. The longer the period on the butter yellow-rice diet, the greater the incidence of resultant liver cancer.

3. Liver cirrhosis produced by butter yellow has been treated successfully by a rice diet containing 15 per cent yeast.

4. Once adenomatous hyperplasia of bile ducts, cholangioma, or hepatoma had been established in the liver, these benign and malignant tumors could not be destroyed by ingestion of the rice-yeast diet.

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The Genesis and Growth of Tumors*

II. Effects of Caloric Restriction per se†

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In a previous communication (7) it was shown that the restriction of food intake of experimental groups of mice resulted in the formation of far fewer tumors than were formed in the corresponding control groups which were fed ad libitum. Moreover, the tumors of the restricted animals appeared at a later time. This inhibition of tumor formation was obtained with all types of tumors studied: spontaneous tumors of breast and lung, and carcinomas and sarcomas induced by a carcinogenic hydrocarbon. The diet given to the experimental groups was restricted in caloric value and correspondingly in all components. Yet the underfed animals maintained their general health, did not reveal any clinical manifestation of nutritional deficiencies, and lived longer than the controls. Deficiencies in vitamins and minerals are not the factors responsible for this observed inhibition of tumor formation; experiments (7) in which the restricted diet was supplemented with vitamins and minerals to the approximate level present in the control diet resulted in the same inhibition. For these reasons, it was stated that the inhibition of tumor formation was "probably due to caloric restriction." At that time decisive experiments to test this deduction were begun. The studies reported in this paper indicate that caloric restriction *per se* is the principal factor in the observed inhibition of tumor formation.

METHODS

In all experiments pure strain mice,[1] bred in our laboratory, were used. They were divided into groups of 50 mice, born within a span of a few weeks. The 2 groups that comprised the experiment were equivalent as to age, weight, and sex; most of the animals in the control and experimental groups were litter mates. Each animal was numbered and a separate record of its progress was kept. The controls were housed 5 in a cage. Each group of 5 mice on the restricted diet was kept in 2 cages; at the biweekly weighings the lighter animals were placed in one cage, the heavier in the other. Thus, the restricted animals competed with others of the same order of weight and, over the long period of the experiment, consumed approximately equal quantities of food and maintained their weights at a fairly constant level.

At the time of weighing the animals were inspected for tumors. When the tumors appeared with peak frequency they were counted weekly. Animals bearing skin carcinomas or subcutaneous tumors were not weighed. The mice were examined postmortem when the tumors became large, at death, or at the termination of the experiment. The lesions were recognized as tumors by their appearance and progressive growth; the type of tumor was established by gross examination and sectioning. Histological examinations were made of many tumors selected at random and of all those lesions about which doubt existed; the results of the histological studies indicated that the gross examinations were reliable. Percentages of tumor formation were computed on the basis of the number of animals alive at the time the first tumor appeared in the experiment (effective total).

The diets were formulated with two objectives: (a) that the control diet be adequate for growth, and (b) that the restricted diet be restricted in calories only, but contain the same amount, at least, of noncarbohydrate components (proteins, fats, vitamins, and minerals) as the control diet. Since in our original studies the restricted animals consumed less noncarbohydrate components than the controls, it seemed advisable to have the restricted animals of this series consume slightly more of these components than their controls; from a practical viewpoint equality in noncarbohydrate components can only be approximated.

* In the first study the term "initiation" was defined and used to imply "the conversion of normal tissue to malignant tissue without consideration of the intermediary phases." Since then, the work of Rous and his associates (4, 6) and of Berenblum (1), as well as our own work (unpublished), clearly indicates that "genesis" or "formation" is a better term for the over-all changes that result in a perceptible tumor. Therefore, the words "initiation" and "inception" will be applied only to those transformations that render cells neoplastic, without consideration of subsequent changes.

† This investigation was aided by a grant from the National Cancer Institute.

[1] The original stock was obtained from the Roscoe B. Jackson Memorial Laboratory.

Both the control and experimental groups were fed equal amounts of a basic ration consisting of 60 per cent Purina dog chow meal and 40 per cent skimmed milk powder. In addition, the diet of the control group contained sufficient cornstarch to complete caloric needs. Thus, both diets contained equal quantities of protein, fat, vitamins, and minerals. The additional carbohydrate in the ad libitum diet results in the approximate percentage compositions shown in the following table:

	Composition of diets	
	Control: ad libitum, per cent	Calorie-restricted, per cent
Protein	16	26
Fat	2	3
Ash	4	7
Carbohydrate	69	53

The exact amounts of food fed and consumed varied, depending on the strain and sex of the mice. In each experiment, however, the control and restricted groups were given the same amount of noncarbohydrate components. The exact diet and food consumption values will be presented under each experiment.

The weighed dietary constituents were mixed with sufficient water to form an easily molded mash, cut into equal blocks, and fed daily. The mice on the restricted diet consumed all the food given them. The actual food consumption of the ad libitum control group was ascertained by weighing back, each week, the food left in the cages. This method of determining food consumption is relatively accurate since analysis of water content of this unconsumed food proved it to be of the same order as that of the original dry food mixture. The amount of food consumed by the control groups in each experiment was relatively constant throughout the experiment.

RESULTS

Effect of a Calorie-Restricted Diet on the Genesis of Induced Skin Tumors

The 2 groups used in this experiment, each consisting of 50 dba male mice, were placed on their respective diets when they were approximately 10 weeks of age. The diets in grams per mouse per day were as follows:

	A0: ad libitum control, gm.	A5: calorie-restricted, gm.
Dog chow meal	1.4	1.4
Skimmed milk powder	0.9	0.9
Cornstarch	1.9	0.0
	4.2	2.3
Average daily food consumption	3.8–4.1	2.3

Four weeks after the diets were instituted, application of the carcinogen was begun. 3,4-Benzpyrene in a 0.3 per cent benzene solution was used, and twice weekly one drop of the solution was applied to the interscapular area by means of a dropping pipette; each mouse was given 19 applications of the carcinogen.

Tumors were recorded as papillomas or carcinomas, but as most of the papillomas eventually became carcinomas and as the exact time of conversion was not always recognizable, the tumor count and time of appearance refer to the first perceptible tumor that each mouse developed.

Tumors appeared in fewer mice, and, on the average, at a later time in the calorie-restricted group (A5) than in the ad libitum control group (A0). Table I summarizes the results of this experiment and in Fig. 1 the cumulative tumor count is presented graphically. The incidence of tumors in the restricted group was 22 per cent as compared with 65 per cent in the ad libitum controls. The mean time of appearance [2] in weeks following the initial application of the carcinogen was 38.0 ± 4.2 and 28.3 ± 2.3 for the tumors of the restricted and ad libitum groups respectively. It should be noted that during the principal course of the experiment there were more tumor-free animals in the restricted group than in the control group.

Effect of a Calorie-Restricted Diet on the Genesis of Induced Sarcomas

The 2 groups, each consisting of 50 C57 black female mice, were placed on their respective diets when they were approximately 11 weeks of age. The diets in grams per mouse per day were as follows:

	N12: ad libitum controls, gm.	N15: calorie-restricted, gm.
Dog chow meal	1.2	1.2
Skimmed milk powder	0.8	0.8
Cornstarch	1.4	0.0
	3.4	2.0
Average daily food consumption	2.6–3.0	2.0

Five weeks after the diets were begun each animal received a single subcutaneous injection in the interscapular area of 0.2 mgm. of 3,4-benzpyrene in 0.2 cc. of lard.

The results of the experiment are shown in Table II and Fig. 2. The restricted animals of N15 developed fewer tumors and, on the average, at a later time than the ad libitum mice of the N12 controls. Forty-four per cent of the animals developed sarcomas in the restricted group, in contrast to 74 per cent in the

[2] The time of appearance was computed from complete data.

TABLE I: THE EFFECT OF A CALORIE-RESTRICTED DIET ON THE FORMATION OF INDUCED SKIN TUMORS IN DBA MALE MICE *

Weeks after first application *	A0: ad libitum controls			A5: calorie-restricted		
	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count
†	23	50	0	22	50	0
0	27	50	0	20	50	0
8	31	49	0	22	50	0
16	34	41	8	21	50	0
24	33	34	15	21	47	2
32	33	26	21	21	39	5
40	32	19	25	22	37	6
48	32	10	29	22	33	9
56	—	4	32	21	29	11
60	—	4	32	20	26	11

* Nineteen applications of benzpyrene solution beginning Sept. 7, 1940.
† Diets started Aug. 10, 1940, 4 weeks before first application of carcinogen.

TABLE II: THE EFFECT OF A CALORIE-RESTRICTED DIET ON THE FORMATION OF INDUCED SARCOMAS IN C57 BLACK FEMALE MICE *

Weeks after injection *	N12: ad libitum controls			N15: calorie-restricted		
	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count
†	20	50	0	21	50	0
0	24	50	0	18	49	0
8	28	50	0	19	48	0
16	30	48	2	20	47	1
24	31	26	24	20	42	6
32	33	17	33	20	29	18
40	36	14	36	21	27	20
48	31	12	37	19	24	21
56	29	12	37	19	23	21
60	—	8	37	20	23	21

* Single injection of benzpyrene on Aug. 1, 1940.
† Diets started June 24, 1940, 5 weeks preceding injection.

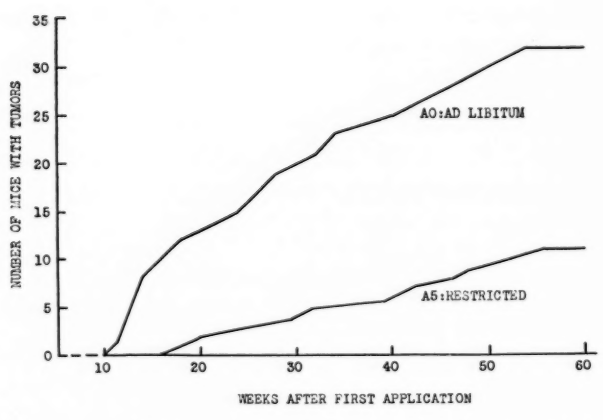

FIG. 1.—Inhibition of the formation of induced epithelial tumors by means of a calorie-restricted diet. Curve of cumulative number of tumors. Time in weeks after first application of carcinogen.

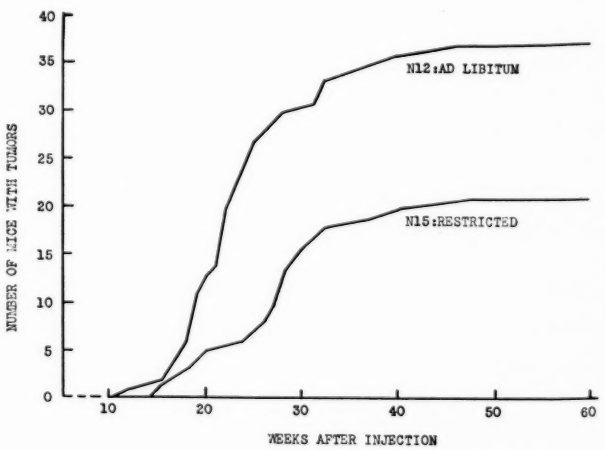

FIG. 2.—Inhibition of the formation of induced sarcomas by means of a calorie-restricted diet. Curve of cumulative number of tumors. Time in weeks after single injection of carcinogen.

ad libitum controls. The mean time of appearance in weeks following the injection of the benzpyrene solution was 27.8 ± 1.6 and 24.0 ± 1.2 for the sarcomas of the restricted and ad libitum groups respectively.

The general health of both groups was excellent until the 46th week. At that time skin ulcerations began to appear in the surviving control animals, prompting the termination of the experiment at 60 weeks by the sacrificing of the remaining control animals. None of the calorically restricted mice were ulcerated; they have been allowed to live and at the present time are in their 73rd week following benzpyrene injection; no new tumors have appeared and 21 animals are still alive. As in the experiment with induced skin tumors, during the principal course of this experiment there were more tumor-free animals alive in the restricted group.

The growth rates of some of the tumors formed in both groups were measured. The growth indices[3] were determined according to the method described in the earlier study (7). Twelve tumors in the N15: restricted group had a mean growth index of 9.8 ± 1.02 in comparison with 12.3 ± 1.02 for 16 tumors of the N12: ad libitum group. The indices ranged from 6.0 to 18.0 and 5.5 to 18.8 respectively. Thus, the average growth rate of the measured tumors in the restricted groups was somewhat less. However, the observed difference in the average growth rates is not significant.

[3] Growth index $= \frac{\text{Change in size of tumor}}{\text{Interval in days}} \times 10.$

Size of tumor = Length plus breadth in millimeters as measured by calipers; estimated error less than 10 per cent.

The Effect of a Calorie-Restricted Diet on the Genesis of Spontaneous Breast Tumors

The dba mice (2 groups of 50) used in this experiment were born in our laboratory within a span of 10 days. After weaning, they were fed Purina dog chow checkers, and at approximately 10 weeks of age they were placed on the experimental diets, which were the same as those utilized in the preceding experiment with induced sarcomas. The average daily consumption of the ad libitum control group, N42, was 2.9 to 3.1 gm.; that of the restricted group was 2.0 gm.

Table III: The Effect of a Calorie-Restricted Diet on the Formation of Spontaneous Breast Tumors in dba Virgin Female Mice

	N42: ad libitum controls			N45: calorie-restricted		
Age in weeks	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count
10 *	20	50	0	21	50	0
40	28	48	0	19	50	0
48	30	47	1	19	50	0
56	30	45	2	20	49	0
64	31	40	6	21	48	0
72	30	35	11	20	46	0
80	29	27	16	20	41	0
86	29	22	18	19	39	0

* Animals placed on experimental diets at 10 weeks of age, Aug. 10, 1940.

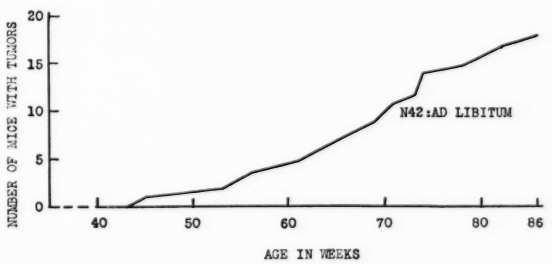

Fig. 3.—Cumulative number of spontaneous breast tumors in ad libitum group of dba virgin female mice. No tumors have appeared in the calorie-restricted group.

The average daily consumption of the ad libitum control group, N42, was 2.9 to 3.1 gm.; that of the restricted group was 2.0 gm.

At the present time the mice are 20 months of age. Eighteen tumors (38 per cent) have appeared in the ad libitum group, while not a single tumor has formed in the restricted group. The results are shown in Table III and Fig. 3.

In the earlier studies concerned with the effect of underfeeding on spontaneous breast tumors in dba mice, we continued the experiments until all the animals died. Although a few tumors arose in the restricted groups at a very late age (23 to 25 months) they in no way altered the results and conclusions of the experiments.

Comparison of the Effects of Restricted Diets Begun at Different Ages

It is of interest to compare the results of 3 studies, all on formation of spontaneous breast carcinomas in dba mice: In all 3 experiments the restricted diets were calorie-restricted, 2 of them containing less and

one somewhat more of noncarbohydrate components than the corresponding control diets. These differences in basal components did not alter the inhibitory effect on tumor formation, as is shown in this communication. The 3 experimental groups differed as to the average age at which they were placed on the restricted diet, and yet, in each case, a marked inhibition of tumor formation occurred. The details of the experiments and the tables have already been presented here and in the earlier paper (7), and therefore they are only summarized in Table IV.

It is obviously unnecessary that caloric restriction be instituted at a very early age in order that breast tumor formation be inhibited. In the first experiment listed in Table IV, the animals were, on the average, 9 months of age before dietary restriction was begun, yet there was a definite and significant decrease in the incidence of spontaneous breast tumors.

Table IV: Effect of Institution of Restricted Diets at Different Ages on the Formation of Spontaneous Breast Tumors in DBA Mice

Experimental groups		Number of mice in experimental groups	Experimental diets started at average age, months	Tumors at 20 months of age	
Restricted	Control			Restricted, per cent	Control, per cent
F15	F12	44	9	5	25
F25	F32	50	5	2	40
N45	N42	50	2	0	38

These experiments suggest that the inhibition of the formation of spontaneous breast tumors of the mouse can be effected if caloric restriction is instituted at any time before the tumors begin to appear. This general view is substantiated by recently completed experiments with tumors of the skin induced by a carcinogenic hydrocarbon.

Résumé of Experiments with Calorie-Restricted Diets

Table V is a résumé of our many experiments dealing with the effects of a calorie-restricted diet on the incidence of tumors, time of their appearance, and longevity of the animals. For simplicity, the data are given as the comparison between the results in the restricted group and the results in the corresponding ad libitum group. "Index of per cent tumors" is the ratio of the percentage of tumors in the restricted group to the percentage of tumors in the control group. A value less than unity indicates that fewer tumors were formed in the restricted group. "Index of mean time of appearance" is the ratio of the average time of appearance of the tumors in the restricted group to the average time of appearance in the controls. A value greater than unity indicates that the tumors of the restricted group appeared at a later time, on the average, than did those of the control group. "Index of longevity" is based on the following considerations: More tumors, and therefore more deaths, occurred in the ad libitum control groups than in the restricted groups. A rigorous method for comparing longevity should therefore exclude the tumor-bearing animals. Such a measure is given by the number of nontumor animals alive at the end of the experiment, expressed as a percentage of all animals which failed to develop tumors. "Index of longevity" is the ratio of this tumor-free survival percentage for the restricted group to that of the percentage for the control group. When the index is greater than unity it indicates that a smaller proportion of the nontumor animals in the restricted group died during the course of the experiment.

In some experiments the figures may differ slightly from those previously presented (7), since at that time the experiments had not been completed. Most of the earlier experiments were discussed in detail in the previous publication. They were performed with a diet restricted proportionally in both calories and components. Groups K19 and K9 differ from the others in that the diet of the former was fortified with vitamins (cod liver oil, brewer's yeast, and wheat germ oil) to approximately the level of the ad libitum control diet, and the latter was fortified with both vitamins and minerals to approximately the level of the ad libitum control diet. In the experiments detailed in the present paper the mice were fed a calorie-restricted diet that contained the same amounts, at least, of the noncarbohydrate components present in the ad libitum control diet.

Table V emphasizes the consistency of the results obtained in the many experiments. It should be noted that in all experiments fewer tumors are formed in the restricted groups and that these tumors appear, on the average, at a later time. In general, the restricted mice live longer than the ad libitum controls. The extent of inhibition in these various experiments differs; however, it is our opinion that these differences are of degree and not of kind, and that they can be explained on the basis of caloric differentials, dosage of and time of exposure to the tumor-producing agent, and difference in strain.

TABLE V: A SUMMARY OF THE EFFECTS OF CALORIE-RESTRICTED DIETS ON THE INCIDENCE OF TUMOR FORMATION, MEAN TIME OF APPEARANCE OF TUMORS, AND LONGEVITY

Values are index numbers of results in the restricted group, based on results in the corresponding control group being 1.00

Type of tumor	Strain of mice	Group number of restricted mice	Index of per cent tumors *	Index of mean time of appearance *	Index of longevity *
Induced epithelial	ABC	D70	0.42	1.9	4.6
	Swiss	K25	0.73	1.2	1.3
	Swiss	K9 †	0.65	1.2	1.0
	Swiss	K19 ‡	0.51	1.2	1.1
	dba	A5 §	0.34	1.4	2.8
Induced sarcoma	C57 bl.	G125	0.68	1.1	0.7 ‖
	Swiss	L5	0.45	1.2	2.0
	C57 bl.	N15 §	0.59	1.2	1.4
Spontaneous breast carcinoma	dba	F15	0.36	1.3	5.7
	dba	F25	0.16	1.3	3.0
	dba	N45 §	0.0	—	1.1
Primary lung tumor	ABC	D70	0.52	..	..
	ABC	F5	0.55	..	..
	Swiss	K9 †	0.17	..	..
	Swiss	K19 ‡	0.21	..	.

* Column headings described in text.

† Not reported previously. Restricted diet fortified with vitamins and minerals to level of ad libitum control.

‡ Not reported previously. Restricted diet fortified with vitamins to level of ad libitum control.

§ The calorie-restricted diet of these groups contained the same amount, at least, of noncarbohydrate components as did the control diet. All other experiments listed had calorie-restricted diets with both calories and components restricted proportionately.

‖ Epidemic of chronic virus pneumonia was more severe in the restricted group.

DISCUSSION

The formation of the induced epithelial tumor, induced sarcoma, and spontaneous breast tumor of the mouse is definitely inhibited by a diet restricted in caloric content. Not only were there fewer tumors in the restricted groups, but those tumors appeared, on the average, at a later time than the tumors of the corresponding ad libitum groups.

As in the earlier publication, it is important to stress the excellent condition of the animals consuming the restricted diet. They were active and sleek and distinguishable from the controls only in that they were somewhat smaller, ranging from 20 to 23 gm. in average weight.

The mice on the calorie-restricted diet outlived the controls. One might expect this result since more animals in the full-fed groups developed tumors and died. Therefore, the fact that the nontumor restricted animals have a longer life span than the nontumor controls is of greater significance and proves the importance of caloric restriction to longevity. This is brought out in Table V, where it can be noted that the number of nontumor mice alive at the end of the experiments, expressed as a percentage of all animals which failed to develop tumors, is greater in the calorie-restricted groups.[4]

Postmortem examination revealed a contrasting appearance of the organs of the mice in the 2 groups. The fat depots of the calorie-restricted mice were definitely depleted. The liver, kidneys, spleen, heart, lungs, and other organs revealed far fewer pathological changes. The gall bladder was distended 2 to 3 times its normal size.

There are some interesting observations regarding ulcerations of the skin. These occurred mainly in the C57 black mice fed ad libitum, and never in any of the animals consuming the restricted diet. In some scout experiments it was noted that although the ulcers of the full-fed mice tended to remain chronic, they generally healed when the animals were placed on the restricted diet. We have not fully investigated this possibly important relationship.

IS INHIBITION OF TUMOR FORMATION CAUSED BY CALORIC RESTRICTION PER SE?

In our earlier work (7), tumor formation was inhibited by a diet restricted in essential components, such as protein, vitamins, minerals, and fats, as well as in calories. It was stated that the effect was probably due to caloric restriction rather than to the proportionate decrease in essential components.

In the numerous studies completed in this laboratory dealing with the effect of a restricted diet on the genesis of tumors there occurred in each experiment a significant inhibition of tumor formation. The experimentally restricted groups always con-

[4] This is in general agreement with the work of McCay and his associates on rats (5).

sumed a diet that was calorie-restricted; in the previously reported experiments they consumed less essential dietary components, while in the present studies they consumed a somewhat larger amount than the corresponding controls. Thus, within the limits of our experiments, and at the general level of underfeeding we have employed, there is inhibition of tumor formation by a calorie-restricted diet, regardless of whether the restricted mice are given less or somewhat more essential dietary components than the corresponding control full-fed mice. Within the limits of our present knowledge, it is evident that caloric restriction *per se* is the principal cause of the observed inhibition of tumor formation.

McCay and his associates (5), in a study on rats retarded by a calorically restricted diet, reported, as an incidental finding, limited evidence indicating a lower incidence of spontaneous tumors in the retarded animals.

Recently, Visscher and his associates (9), using one of the tumors we have studied, the spontaneous breast carcinoma of the mouse, have confirmed our results and our deduction that the inhibitory effect was due to caloric restriction; by means of a calorie-restricted diet they reduced the incidence of breast tumors in the C3H strain from 67 to 0 per cent. We are in agreement that this effect must be due to caloric restriction.

Mechanism of Tumor Inhibition Caused by a Calorie-Restricted Diet

Considering the present knowledge of carcinogenesis it is obvious that any attempted explanation of the inhibition of tumor formation through a calorie-restricted diet must be hypothetical.

Emphasis must again be placed on the fact that inhibition was obtained with various types of tumors, such as the spontaneous carcinoma of the breast, spontaneous tumor of the lung, induced epithelial tumor, and the induced sarcomas. Also, different strains of mice and both sexes were employed. All this suggests that the intermediary factors causing this inhibiting effect are general in nature (possibly an effect on the whole endocrine-metabolic complex), present in all the tissues of the body, and effective at all sites investigated. For example, in a previous investigation (7) the same control and restricted groups of mice were utilized to study the effect of a restricted diet on two entirely different types of tumors, the induced epithelial tumor and the spontaneous tumor of the lung. The carcinogenic stimuli responsible for the genesis of these two tumors must certainly be different,[5] yet the formation of both types was inhibited by a restricted diet.

[5] The experimental data show that the application of the carcinogenic hydrocarbon to the skin, in the amounts used, had no effect upon the percentage of spontaneous lung tumors.

It seems unlikely that reduction in the normal out put of hormones by the ovary is the responsible factor for the observed inhibition of spontaneous breast tumors. In our previous studies (7) we came to the conclusion that the effects due to dietary inhibition of the ovaries were probably minimal. This opinion was based on the following reasoning: The classic work of Loeb (2, 3) has shown that extirpation of the ovaries of 7- to 9-month-old mice does not reduce the incidence of breast tumors, indicating that the breast had already been conditioned. Yet a restricted diet, begun when mice were 9 months old, was able to inhibit the development of breast tumors. This strongly suggests that the retarding effects cannot be due to lack of ovarian hormone reaching the breast, and also that the inhibitory factors exercise their principal effect during the developmental or formative stage rather than during the stage of initiation. Our experiments with induced tumors, where the active agent was directed to the site of tumor formation, confirms our view that the main inhibiting effects are not due to a reduction in the amount of carcinogenic agent reaching the site of tumor formation.

The experimental evidence supports a very general hypothesis as to the mechanism of tumor inhibition by means of a calorie-restricted diet: The restricted diet limits the nutritive supply, metabolism, and growth of the tissue that is or has been subjected to carcinogenic stimuli; under such conditions tumor formation is inhibited. It may be that initiation of a tumor occurs even with a restricted nutritive supply, but that energy over and above a basic level is required before a perceptible tumor can eventuate. Or, it may be that an animal on a calorie-restricted diet utilizes a greater proportion of essential components (protein, vitamins, etc.) for maintenance energy, and therefore these are not present in sufficient quantity to permit the genesis of the tumor.

Significance

The inhibition of tumor formation by means of a calorie-restricted diet, observed in the study of four varied types of tumors, is probably of general significance. This does not imply, however, that all neoplasms of all species will behave in a similar manner.

From the viewpoint of experimental cancer research, the results obtained have importance beyond the generalization that caloric restriction, in the presence of maintenance amounts of noncarbohydrate constituents, inhibits the formation of tumors. They imply that any experimental procedure that produces secondarily a restricted food intake or assimilation may produce results that might be mistaken for a primary effect. For example, an experiment may be reported in which there is observed an inhibition of tumor formation. It is also stated that the animals were debilitated or

failed to grow. It is our opinion that before the inhibition of tumor formation is ascribed specifically to the particular experimental procedure employed, the very general effect of caloric restriction must be ruled out. It is suggested that in such experiments careful consideration be given to the diet, food consumption, and body weight, in comparison with normal values.

The possibility that the experimental results have practical value with regard to human cancer has been discussed in detail (8) in a review of insurance statistics concerned with the relationship of body weight to cancer incidence. It is shown that persons of average weight or less are not as likely to develop cancer as those who are overweight. It follows that the avoidance of overweight through restriction of food intake may aid in the prevention of human cancer or at least delay its onset.

That spontaneous breast carcinoma of the mouse may be prevented by a restricted diet, even one started at a period well along in the life span of the animal, suggests that a controlled and calorie-restricted diet for human beings may be of value at any time before the possible onset of a tumor.

SUMMARY

1. By utilizing the spontaneous breast tumor, induced carcinoma, and induced sarcoma of the mouse, the effects of a calorie-restricted diet on the genesis and growth of tumors were studied. The average weight of the restricted mice was 20 to 23 gm.

2. The calorie-restricted diet inhibited the formation of tumors. This inhibition involved both a decrease in the total number of tumors and a delay in the time of appearance.

3. The principal factor in this inhibition of tumor formation was found to be caloric restriction *per se*.

4. Mice on a calorie-restricted diet appeared healthy and, in general, outlived the ad libitum control mice. This statement is valid even when only those animals which do not develop tumors are considered.

5. The average growth rate of sarcomas which formed in mice on a calorie-restricted diet was not significantly less than that of tumors which appeared in the ad libitum controls. This is in agreement with our previous results with spontaneous breast tumors.

6. Inhibition of formation of spontaneous breast tumors can probably be effected if caloric restriction is instituted any time before the tumors begin to appear. Definite inhibition occurred whether the mice (dba) were 2, 5, or 9 months of age at the time the calorie-restricted diet was begun.

7. A mechanism is suggested for the inhibitory effect due to the calorie-restricted diet. The significance of the conclusions of this communication in relation to experimental cancer research and human cancer is discussed.

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The Genesis and Growth of Tumors

III. Effects of a High-Fat Diet*

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Watson and Mellanby (10) have found that feeding mice a diet containing 12.5 to 25 per cent butter fat causes a definite increase in the incidence of skin tumors produced by tarring. The results of Baumann and his associates (1, 2, 5, 6) are in the same direction. In numerous experiments they have shown that skin tumors of the mouse, induced either by ultraviolet light or carcinogenic hydrocarbons, are formed in greater numbers and at an earlier time in mice receiving a high-fat diet than in control mice consuming the basal rations. In contrast to these results, the same investigators have found that the production of sarcomas by carcinogenic hydrocarbons is not significantly altered by a high-fat diet.

This communication is a report of the effects of dietary fat on both the genesis and growth of tumors. The tumors utilized were the spontaneous breast tumor, induced skin tumor, induced sarcoma, and primary lung tumor of the mouse. The investigations demonstrate that increasing the fat content of a basic ration exerts diverse effects on the formation of different types of tumors; these effects range from a striking augmentation of the formation of spontaneous breast tumors to a possible inhibition of the formation of sarcomas induced by a carcinogenic hydrocarbon. An attempt has also been made to clarify the mechanism by which fat affects the genesis of tumors.

METHODS

In all experiments pure strain mice were used, obtained from the Roscoe B. Jackson Memorial Laboratory or derived from their stock. They were divided into groups equivalent as to age, weight, and sex, usually 50 for the experimental group and 50 for the control group. Each animal was numbered and a separate record of its progress was kept. The animals were inspected for tumors biweekly, at which time they were weighed unless they bore skin carcinomas or subcutaneous tumors. Postmortem examination was performed when the tumors became large, at the death of the animal, or at the termination of the experiment. The lesions were recognized as tumors by their appearance and progressive growth; the type of tumor was established by gross examination and sectioning. Histological examinations were made of many tumors, selected at random, and of all those lesions about which doubt existed; the results of the histological studies indicated that the gross examinations were reliable. Percentages of tumor formation were computed on the basis of the number of animals alive at the time the first tumor appeared in either group of the experiment (effective total). The "tumor count" refers to the number of animals which developed tumors.

All animals were fed ad libitum and had free access to water. In each experiment the control group was fed a basic ration relatively low in fat, but adequate for growth, while the second group was fed the same basic ration modified by the substitution of fat.[1] Two basic rations were employed, modified in such a way that 3 experimental high-fat diets resulted. The diets were prepared by mixing the dry components with sufficient water to form an easily molded mash, which was cut into equal blocks, each containing a definite amount of the dry mixture. The actual average food consumption per animal was obtained each week by weighing back the food left in the cages. These values were not obtained in our earlier experiments.

Diet 1.—The control diet (1c) was a basic ration consisting of cracked spring wheat, 145; Purina dog chow meal, 40; skimmed milk powder, 15; and white milled flour, 25. The high-fat diet (1f) was prepared from the basic ration by substituting 25 parts of fat for 25 of wheat, and 5 parts of vitamin-free casein for 5 of flour. On Sunday an equivalent amount of Purina dog chow checkers was fed to both the control and high-fat groups. The approximate compositions of the diets were as follows:

	Control (1c), per cent	High-fat (1f), per cent
Protein	17	17
Fat	3	12
Carbohydrate	64	58
Ash	3	3

* This investigation was aided by a grant from the National Cancer Institute.

[1] Hydrogenated cottonseed oil (kremit), generously furnished by Armour and Company.

Diet 2.—The control basic ration (2c) was the same as in diet 1. The high fat diet (2f) was made by substitution, in a manner similar to that used in preparing high-fat diet 1f, but differing in that 75 parts of fat replaced 50 of wheat. Otherwise, the method of preparation of the diets, daily feeding, and Sunday feeding were the same as with diet 1. The approximate compositions of the diets were as follows:

	Control (2c), per cent	High-fat (2f), per cent
Protein	17	15
Fat	3	28
Carbohydrate	64	46
Ash	3	3

Diet 3.—Experience and refinement of technic resulted in diet 3. The control diet (3c) consisted of 1.4 gm. Purina dog chow meal, 0.9 gm. skimmed milk powder, and 1.9 gm. cornstarch. This amount was fed daily to each animal. The high-fat diet (3f) was prepared by substituting an isocaloric amount (0.9 gm.) of hydrogenated cottonseed oil for the 1.9 gm. of starch. Thus equicaloric amounts of the 2 diets contained equal quantities of protein, vitamins, and minerals, and differed only in the fat and carbohydrate content. The approximate compositions of the diets in grams per mouse per day were as follows:

	Control (3c), gm.	High-fat (3f), gm.
Protein	0.62(15%)	0.62(19%)
Fat	0.08(2%)	0.98(31%)
Carbohydrate	2.92(70%)	1.22(38%)
Ash	0.16(4%)	0.16(5%)

It is to be noted that the fat content of the 3 high-fat diets was 12 to 31 per cent in comparison with a fat content of 2 to 3 per cent for the 2 control rations.

RESULTS

Effects of a High-Fat Diet on the Formation of Spontaneous Breast Tumors

Experiment 1.—The control and experimental groups were each composed of 44 female dba mice, matched as to their birth dates, number of litters, and litter dates (12 in each group were virgin). At an average age of 38 weeks (range, 32 to 48 weeks) they were placed on their respective diets: diet 1c, control, and the corresponding high-fat diet (1f) described under "Methods." The experiment was continued until all the animals had died. There was no observable difference in the general health and appearance of the mice in the 2 groups, and the rate of nontumor deaths was approximately the same in both groups.

The results as shown in Table I and Fig. 1 A reveal that more tumors were formed in the high-fat group. Twenty-four tumors (55 per cent) arose in the F11 group receiving the high-fat diet in contrast to 14 tumors (32 per cent) in the F12 group fed the basic diet. The tumors in the high-fat group arose at a mean age of 70 ± 3.1 weeks in comparison with 72 ± 5.7 weeks for those of the control group.

Experiment 2.—Experiment 1 was repeated on 2 groups of 50 virgin dba mice. At an average age of 24 weeks (range, 18 to 32), they were placed on the control (1c) and high-fat (1f) diets. The experiment was terminated when the mice had attained an average age of 2 years, at which time only 3 of the control group (F22) and 2 of the high-fat group (F21) were alive and without tumors.

The results are shown in Table II, and the cumulative tumor counts are graphically represented in Fig. 1 B. Thirty-two spontaneous breast tumors (64 per cent) arose in the high-fat group in comparison with 16 tumors (32 per cent) in the control group. The tumors in the high-fat group appeared at a mean age of 62 ± 1.8 weeks, compared with 74 ± 3.1 for those of the control group. Thus, the high-fat diet caused the formation of twice as many tumors and a significant shortening of the mean age of appearance. As in experiment 1, there was no observable difference in the general health and appearance of the 2 groups, and the rates of nontumor deaths were approximately the same.

The definite increase in the incidence of spontaneous breast tumors brought about by a fat-enriched diet is significant, and this effect is the most striking result obtained in our studies with various types of tumors. Also, the tumors appeared at an earlier time. There are certain quantitative differences in the results of the 2 experiments. The control groups of both experiments had 32 per cent tumors while the high-fat groups of experiments 1 and 2 had 55 per cent and 64 per cent respectively. Furthermore, in experiment 1, the high-fat diet did not significantly decrease the mean age at which the tumors appeared, while in experiment 2 the mean age of appearance of the tumors was shortened by about 3 months. The mice of experiment 2 were approximately 14 weeks younger than those of experiment 1 at the beginning of these experiments and were, therefore, subjected to the dietary differences for a longer period. It is suggested that if mice be fed a high-fat diet throughout their early adult life a more decided augmentation and acceleration of tumor formation occurs. When the animals of experiments 1 and 2 were classified into smaller subgroups, according to the age at which the diets were instituted, it appeared that the augmentation of tumor formation through the high-fat diet was less pronounced in the older subgroups.

TABLE I: THE EFFECTS OF A HIGH-FAT DIET ON THE FORMATION OF SPONTANEOUS BREAST TUMORS IN DBA MICE

	F12: control, diet 1c			F11: high-fat, diet 1f		
Average * age, weeks	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count
38	29	44	0	28	44	0
46	30	43	1	31	39	3
54	31	40	3	33	36	5
62	31	34	6	34	32	7
70	31	28	8	34	26	9
78	28	24	10	33	19	14
86	28	13	11	32	5	22
94	28	9	11	..	0	24
102	—	2	13	..	..	..
110	..	0	14	..	..	..

* Average age of mice at beginning of experiment (May 17, 1938): 38 weeks.

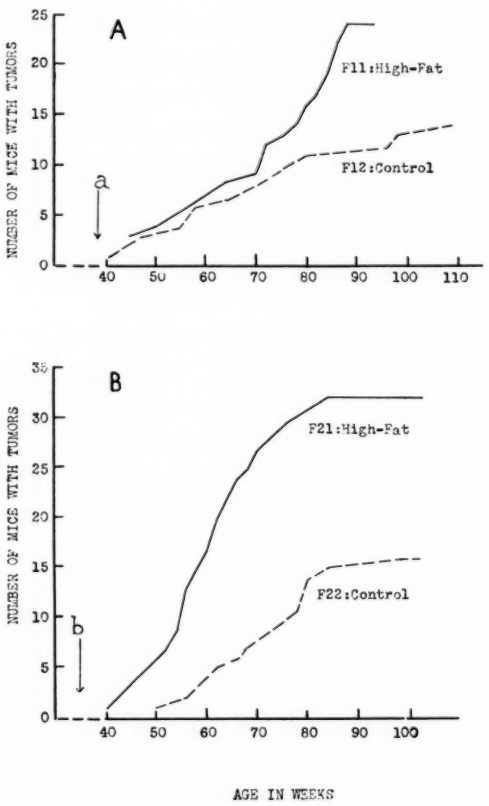

Fig. 1.—Effects of a high-fat diet on the formation of spontaneous breast tumors in dba female mice. Curve of cumulative number of tumors. (a) Diets instituted at 38 weeks. (b) Diets instituted at 24 weeks.

EFFECTS OF A HIGH-FAT DIET ON THE FORMATION OF INDUCED EPITHELIAL TUMORS

These experiments were performed with 3 different strains of mice. The skin tumors were induced by a 0.3 per cent benzene solution of 3,4-benzpyrene. Twice weekly, in general, one drop of the solution, containing about 0.05 mgm. of the carcinogen, was applied to the interscapular region by means of a dropping pipette. Tumors were recorded as papillomas or carcinomas, but since most of the papillomas eventually became carcinomas, and the exact time of conversion was not always recognizable, the tumor counts include both types.

Experiment 3.—Two groups, each containing 45 JAX Swiss females, were placed on their respective control and high-fat diet 2 when they were about 10 weeks of age. They received 32 applications of the benzpyrene solution during the following 20 weeks. The average daily food consumption for the high-fat group (K1) was 3.0 gm. per mouse compared with 3.3 gm. for the control animals (K2). The experiment was continued for 42 weeks following the initial application of the carcinogen.

TABLE II: THE EFFECTS OF A HIGH-FAT DIET ON THE FORMATION OF SPONTANEOUS BREAST TUMORS IN DBA VIRGIN MICE

	F22: control, diet 1c			F21: high-fat, diet 1f		
Average * age, weeks	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count
24	27	50	0	26	50	0
32	30	50	0	31	50	0
40	31	50	0	34	49	1
48	33	50	0	36	44	5
56	34	47	2	38	36	13
64	33	38	5	37	25	20
72	32	26	7	33	15	28
80	31	14	14	34	8	30
88	28	8	15	—	4	32
96	—	5	15	—	3	32
102	—	3	16	—	2	32

* Average age of mice at beginning of experiment (June 30, 1938): 24 weeks.

The results are shown in Table III. Twenty-eight skin tumors (67 per cent) were formed in the high-fat group and 22 tumors (51 per cent) in the control group. The mean time of appearance of the tumors in the high-fat group was 23 ± 1.3 weeks, compared with 24 ± 1.7 for those of the control group.

Experiment 4.—Two groups of 50 C57 black male mice, all born within a span of 3 weeks, were transferred to their respective control and high-fat diet 3 when they were 10 weeks of age. At 16 weeks of age they received the first of 26 semiweekly applications of the benzpyrene solution. The animals of the control group (S0) consumed an average of 3.5 gm. per day in comparison with 3.0 gm. per day for the high-fat group (S1). It is to be noted that these amounts of food contained approximately the same quantities of essential dietary components (protein, vitamins, and minerals) and were approximately isocaloric.

The results are given in Table IV and the tumor counts are graphically presented in Fig. 2 A. In the high-fat group (S1), 17 mice (35 per cent) developed tumors compared with 13 (27 per cent) in the corresponding control group (S0). The mean time of appearance of the tumors in the high-fat group was 31 ± 2.8 weeks, compared with 34 ± 3.0 in the control group. Toward the end of the experiment skin ulcerations occurred in approximately equal numbers in both groups.

Experiment 5.—Two groups of 50 mice each were made up of dba male mice born within a span of 6 weeks. At 10 weeks of age the groups were placed on their respective control and high-fat diet 3. Four weeks later the first of 19 semiweekly applications of the benzpyrene solution was begun. The average daily food intake per mouse was 4.0 gm. for the control group and 3.1 for the high-fat group. Again, as in the previous experiment, the animals were fed ad libitum; yet the 2 groups consumed isocaloric amounts of food containing approximately equal quantities of essential dietary components.

TABLE III: THE EFFECTS OF A HIGH-FAT DIET ON THE FORMATION OF INDUCED SKIN TUMORS IN MALE SWISS MICE

Weeks after first application *	K2: control, diet 2c			K1: high-fat, diet 2f		
	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count
1	21	45	0	20	45	0
11	25	43	0	26	40	2
15	27	37	3	28	36	6
19	29	32	7	30	34	7
23	31	30	8	32	25	15
27	33	21	17	35	16	23
31	33	19	19	35	14	25
35	32	19	19	38	11	27
39	34	16	21	38	10	28
42	35	15	22	39	9	28

* Thirty-two applications of benzpyrene solution beginning March 10, 1939.

The results are shown in Table V and Fig. 2 B. Thirty-nine skin tumors (78 per cent) were formed in the high-fat group, in comparison with 34 tumors (68 per cent) in the control group. The mean time of appearance of these tumors was 27 ± 2.2 and 31 ± 1.8 weeks respectively.

It should be noted from the tables and figures that in the stage of each experiment when only a few tumors had formed (21 to 24 weeks) the percentage difference in the incidence of tumors in the 2 groups was relatively large. However, by the time the experiments were terminated (42 to 56 weeks) larger numbers of tumors had formed in both groups, resulting

TABLE IV: THE EFFECTS OF A HIGH-FAT DIET ON THE FORMATION OF INDUCED SKIN TUMORS IN MALE C57 BLACK MICE

Weeks after first application *	S0: control, diet 3c			S1: high-fat, diet 3f		
	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count
†	24	49	0	24	50	0
0	28	49	0	28	50	0
13	34	49	0	36	50	0
17	35	47	2	38	45	4
21	38	46	2	40	43	5
25	39	43	4	41	40	6
29	38	42	4	42	38	7
33	40	39	6	44	36	9
37	40	35	6	44	31	11
41	39	26	10	42	24	14
45	38	21	11	40	21	15
49	—	19	13	—	15	17

* Twenty-six semiweekly applications of benzpyrene solution beginning Aug. 2, 1940.

† Diets started on June 24, 1940, 6 weeks before first application of carcinogen.

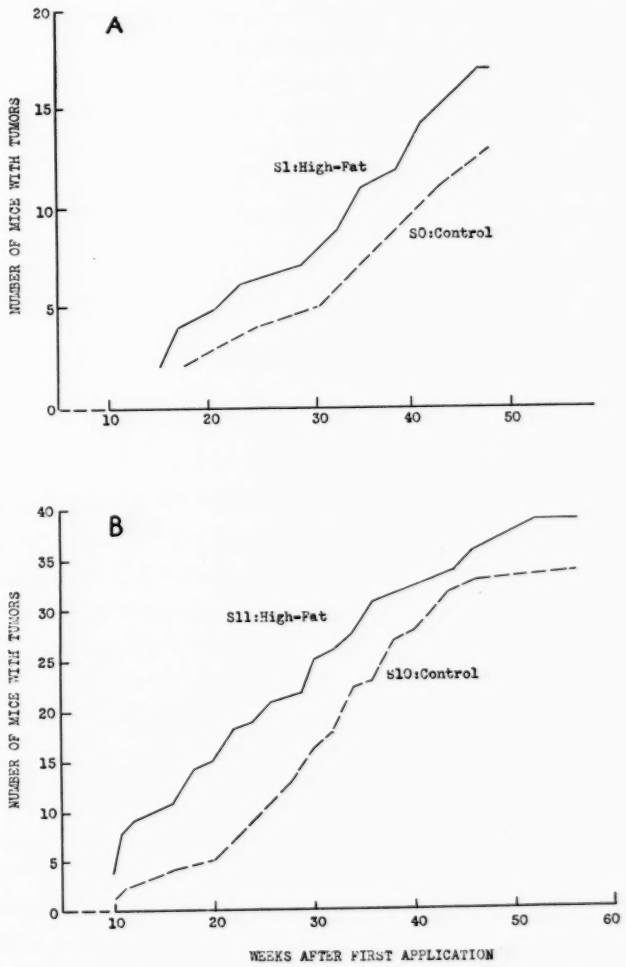

FIG. 2.—Effects of a high-fat diet on the formation of induced epithelial tumors. Curve of cumulative number of tumors.

in smaller relative differences in the incidence of tumors. These facts in no way alter the interpretation of the results, but do indicate that the magnitude of the effect appears to be greater in the early stages of the experiments.

In 2 experiments on JAX ABC mice comparable results were obtained when the fat content of the basic ration was increased by the addition of 10 per cent wheat germ oil, instead of hydrogenated cottonseed oil.

Thus, all 5 experiments with induced skin tumors show the same results: A high-fat diet produced a definite increase in the incidence of skin tumors and shortened the mean time of appearance of these tumors. Although the differences in any one experiment are not of significant magnitude statistically, the results are remarkably consistent and are in qualitative agreement with those obtained by other workers (2, 5, 6, 10).

TABLE V: THE EFFECTS OF A HIGH-FAT DIET ON THE FORMATION OF INDUCED SKIN TUMORS IN MALE DBA MICE

Weeks after first application *	S10: control, diet 3c			S11: high-fat, diet 3f		
	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count
†	23	50	0	23	50	0
0	26	50	0	25	50	0
8	29	50	0	29	50	0
16	32	46	4	31	38	11
24	30	40	9	31	30	19
32	32	31	18	34	23	26
40	31	20	28	32	18	31
48	—	8	33	33	11	36
56	—	3	34	—	7	39

* Nineteen semiweekly applications of benzpyrene solution beginning Sept. 7, 1940.

† Diets started Aug. 10, 1940, 4 weeks before first application of carcinogen.

EFFECTS OF A HIGH-FAT DIET ON THE FORMATION AND GROWTH OF INDUCED SARCOMAS

Experiment 6.—Two groups, each of 40 JAX Swiss female mice 10 weeks of age, were given a single subcutaneous injection of 0.15 mgm. of 3,4-benzpyrene in 0.2 cc. of lard in the interscapular area. At that time they were placed on their respective control and high-fat diet 2. The control group (L30) consumed a daily average of 3.0 gm. per mouse compared with 3.4 gm. for the high-fat group (L1).

Fewer tumors were formed in the high-fat group. The results are given in Table VI and Fig. 3 B. Twelve sarcomas (30 per cent) arose in the high-fat group in comparison with 19 sarcomas (49 per cent) in the control group. The mean time of appearance of the tumors was 25 ± 2.7 and 25 ± 1.4 weeks respectively.

The rate of growth of the tumors in the 2 groups did not differ. The 13 tumors measured in the normal group (L30) had a mean growth index [2] of 13 ± 1.1

TABLE VI: THE EFFECTS OF A HIGH-FAT DIET ON THE FORMATION OF INDUCED SARCOMAS IN FEMALE SWISS MICE

Weeks after injection *	L30: control, diet 2c			L1: high-fat, diet 2f		
	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count
0	19	40	0	19	40	0
8	24	40	0	26	40	0
16	28	37	1	32	38	2
24	30	27	11	36	32	7
32	31	18	18	40	27	9
40	31	17	19	42	21	12
48	34	15	19	43	18	12
52	34	15	19	42	16	12

* Single injection of 3,4-benzpyrene on May 22, 1939.

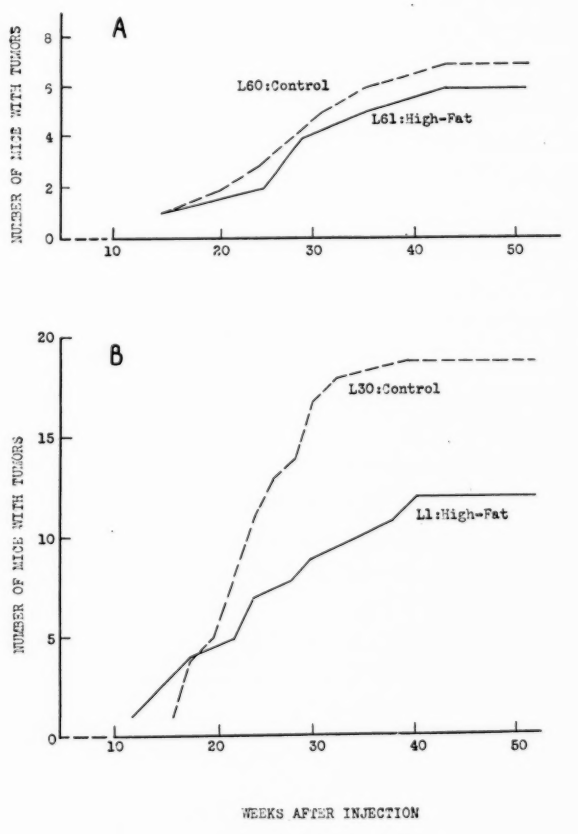

FIG. 3.—Effects of a high-fat diet on the formation of induced sarcomas. Curve of cumulative number of tumors.

[2] Growth index $= \frac{\text{Change in size of tumor}}{\text{Interval in days}} \times 10$

Size of tumor = Length plus breadth in millimeters as measured by calipers; estimated error less than 10 per cent.

compared with 13 ± 2.3 for the 9 tumors measured in the high-fat group (L1).

Experiment 7.—At 9 weeks of age 40 JAX ABC mice were placed in the control group (L60) and 37 mice in the high-fat group (L61). A single subcutaneous injection of 0.1 mgm. of 3,4-benzpyrene in 0.2 cc. of lard was given in the interscapular region. Diets 2c and 2f were again utilized. The daily average food consumption per mouse was 3.6 and 3.3 gm. for the control and high-fat groups respectively.

In Table VII and Fig. 3 A the results of this experiment are given. Six sarcomas (16 per cent) were formed in the high-fat group, 7 (18 per cent) in the control group. The mean time of appearance of these tumors was 29 ± 3.9 and 28 ± 3.5 weeks respectively.

TABLE VII: THE EFFECTS OF A HIGH-FAT DIET ON THE FORMATION OF INDUCED SARCOMAS IN FEMALE ABC MICE

	L60: control, diet 2c			L61: high-fat, diet 2f		
Weeks after injection*	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count	Mean weight, gm.	Animals alive and tumor-free	Cumulative tumor count
0	18	40	0	18	37	0
7	25	40	0	27	37	0
15	32	39	1	34	36	1
23	35	38	2	41	35	1
31	38	33	5	44	31	4
39	39	31	6	45	30	5
47	40	30	7	44	29	6
51	40	29	7	42	25	6

* Single injection of 3,4-benzpyrene on May 27, 1939.

As in the previous experiment, growth rates of tumors were studied. No appreciable difference was found between the mean growth indices of the tumors in the control and high-fat groups: 14 ± 1.3 (6 tumors) and 13 ± 2.7 (4 tumors) respectively.

Thus, a high-fat diet definitely does not increase the formation of induced sarcomas; under selected conditions of carcinogenesis it may even inhibit their formation. The results of these 2 experiments with induced sarcomas are in decided contrast to those obtained with spontaneous breast tumors (considerable increase) and induced epithelial tumors (moderate increase).

EFFECTS OF A HIGH-FAT DIET ON THE FORMATION OF PRIMARY LUNG TUMORS

Three of the experiments reported (experiments 3, 6, and 7) were performed on strains of mice that normally develop primary lung tumors. At the termination of these investigations it seemed expedient to utilize the postmortem examination records to study the effects of the high-fat diet on the formation of primary lung tumors. Primary epithelial lung tumors were found in relatively equal numbers in the control and high-fat groups. However, one must consider that these results were observed in animals which were not much over one year of age, well before the maximum number of lung tumors is expected. The tumors in both groups were, on the average, from 1 to 3 mm. in diameter. Table VIII shows the results of this study. It is evident that a high-fat diet has no significant effect upon the genesis of primary lung tumors. Watson and Mellanby (10) reported that there was a slight increase in the number of lung tumors in their high-fat groups. However, it is difficult to compare our finding with theirs since they considered only lung tumors in animals with tumors of the skin while we considered only those of animals free from other tumors. They stated that the lung tumors in their animals were both metastatic tumors and primary lung tumors, and it is probable that the metastatic tumors were more numerous in the high-fat animals since their skin tumors were formed earlier.

DISCUSSION

The most striking result of these investigations is the diversity of effects produced by a high-fat diet: The incidence of spontaneous breast carcinoma in the mouse was significantly increased; the formation of induced skin tumors was also increased, but probably to a lesser extent; the primary lung tumor incidence was unaffected; and the formation of induced sarcomas was unaffected or actually inhibited. Furthermore, whenever an increase in the incidence of tumors occurred, there was also a shortening of the mean time of appearance. The results, summarized in Table VIII, are the outcome of investigations in which: (a) at least 2 experiments were performed with each type of tumor; (b) various strains of mice were used; (c) adequate numbers of animals were employed; (d) the experiments were continued for a sufficiently long period; (e) the general health of both the control and experimental groups was good; and (f) the number of nontumor deaths was not unusual and was of the same order in both groups.

Some investigators have reported that fat-enriched diets produced a decided greasiness of the skin in their animals. We have noted only the slightest oiliness of the skin in animals on the diets containing 31 per cent fat, and none in the animals on the diet containing 12 per cent fat. The animals on the high-fat diets were heavier, in general, and had a greater proportion of fat, as well as more, in the fat depots (subcutaneous, genital, perirenal, mesenteric, etc.).

The assumption is made that in a qualitative sense the diverse effects of a fat-enriched diet observed in

these experiments are essentially real, and dependent on the type of tumor. There is no reason to believe that fat must act in only one way. It seems to us that the effects reported in this communication may be the resultant of two properties of fat: (a) "solvent action" on the carcinogen; and (b) "cocarcinogenic action" on the developing tumor cell. Under certain conditions solvent action may concentrate the carcinogen in a particular area, while under other conditions it may remove the carcinogen, as from a site of injection. Cocarcinogenic action may be considered to be a

TABLE VIII: THE DIVERSE EFFECTS OF A HIGH-FAT DIET ON THE FORMATION OF DIFFERENT TYPES OF TUMORS

Type of tumor	Group experiment number	Number of mice (effective total)	Tumors, per cent	Mean time of appearance, weeks
Spontaneous breast carcinoma	F12: control	44	32	72 ± 5.7 *
	F11: high-fat	44	55	70 ± 3.1
	F22: control	50	32	74 ± 3.1
	F21: high-fat	50	64	62 ± 1.8
Induced skin tumors	K2: control	43	51	24 ± 1.7 †
	K1: high-fat	42	67	23 ± 1.3
	S0: control	49	27	34 ± 3.0
	S1: high-fat	50	35	31 ± 2.8
	S10: control	50	68	31 ± 1.8
	S11: high-fat	50	78	27 ± 2.2
Induced sarcomas	L30: control	39	49	25 ± 1.4 †
	L1: high-fat	40	30	25 ± 2.7
	L60: control	40	18	28 ± 3.5
	L61: high-fat	37	16	29 ± 3.9
Primary lung tumors	K2: control	15	52	52 ‡
	K1: high-fat	9	44	
	L30: control	15	27	62
	L1: high-fat	16	25	
	L60: control	29	34	60
	L61: high-fat	25	36	

* Mean age of mice.
† Mean time after first application of carcinogen.
‡ Mean age of mice at time of examination.

metabolic stimulation of carcinogenesis. In the light of this hypothesis the effect of a high-fat diet on the genesis of the tumors studied will be discussed.

Spontaneous breast tumors.—It is possible that a high-fat diet increases the formation of spontaneous breast tumors principally through the action of larger quantities of estrogenic hormone held in solution in the larger amounts of adipose tissue surrounding the breasts of mice on a fat-enriched diet. For this type of tumor the solvent action and cocarcinogentic action may act together to produce the augmentation observed.

Induced skin tumors.—According to Beck and Peacock (3) chemical carcinogens disappear from the surface of mouse skin within a few days. Using similar methods we have found that the carcinogen tends to disappear somewhat more rapidly from the surface of the skin of mice that are on a high-fat diet. This suggests that the carcinogen may be carried into the skin more rapidly in such mice. There is at least one fact, however, that argues against solvent action as the cause of the observed augmentation of tumor formation: Experiments in which fat-enriched diets were fed during various phases of the tumor process (6, 8) suggest that fat feeding in the period *following* the application of the carcinogen is more effective in increasing the formation of tumors. This fact, however, is compatible with the view that the increased tumor incidence is due to cocarcinogenic action.

Induced sarcomas.—Baumann, Jacobi, and Rusch (2) carried out experiments with large dosages of carcinogen (0.5 to 1.25 mgm.), obtaining high percentages of tumors in both the control and high-fat groups. There was no significant difference in tumor formation. On the other hand, our experiments were carried out with 0.1 and 0.15 mgm. of 3,4-benzpyrene and resulted in what may be an inhibition of tumor formation. It is possible that this lack of agreement may be due to different dosages [3] of carcinogen.

It is generally believed that the injected carcinogen gradually disappears from the animal's body. It is probably removed from the injected solvent by a partition between the solvent and the subcutaneous tissue (fat) of the animal. Through increased solvent action a given amount of carcinogen, dissolved in a medium such as lard, would probably be removed at a faster rate if injected into subcutaneous tissue containing large amounts of fat (high-fat group) than if injected into subcutaneous tissue of normal animals (control group). Under these conditions a comparatively smaller "effective dose" (in contrast to the amount injected) would remain at the injection site in the high-fat animals, resulting in fewer tumors. This view is given credence since: (a) in our high-fat animals the lard cysts disappeared more rapidly, suggesting that there may also be a more rapid removal of the carcinogen from the injection site; and (b) Peacock and Beck (7) have shown that groups of mice in

[3] It is now generally believed that a dose of carcinogen large enough to produce tumors in practically 100 per cent of the animals employed may mask or override the effect of an experimental procedure. A smaller dose might permit the effect to be disclosed. The effectiveness of a given quantity of carcinogen is dependent on many factors. In referring to a dose as "high" we imply that in a particular experimental procedure this dose produced tumors in practically all the animals; a "low" dose, on the other hand, is one that produces only a small percentage of tumors even when the experiment is permitted to continue throughout the life span of the animals.

which the carcinogen was retained at the site of injection for a shorter time developed fewer tumors.

The difference between the removal rates of carcinogen in the control and the high-fat group may result, depending on the original dose of carcinogen, in diverse effects. It is probable that the rate of removal of carcinogen from an injection site is proportional to the original dose of carcinogen. Bryan and Shimkin (4) have shown that if the percentage of tumors induced by a given dose of carcinogen is plotted against the logarithm of the dose, an ∫-shaped curve is obtained. Consequently, it may be expected that proportionate decreases (due to a high-fat diet) in the effective dosage of carcinogen would result in a lesser effect on tumor incidence when the injected dose is either high or low (at the extremities of the ∫-curve) than when the carcinogen is injected in intermediate amounts (middle of the ∫-curve). This hypothesis would explain the negative results of Baumann, Jacobi, and Rusch (2), in which high dosages were used, the negative results of our own experiment 7, in which a low dosage was used, and the decrease in tumor incidence observed in our experiment 6, in which an intermediate dosage was employed.

Primary lung tumors.—The incidence of this type of tumor is unaffected by a high-fat diet. This may be due to the fact that fat is not deposited in the lung; thus, neither the solvent action nor the cocarcinogenic action of fat would be expected to exert any effect on the formation of primary lung tumors.

Growth of tumors formed in animals on a high-fat diet.—Baumann, Jacobi, and Rusch (2) found that the growth rate of tumors of the ear in mice, induced by ultraviolet light, appears to be unaffected by a high-fat diet. In our experiments with induced sarcomas there was no significant difference in the mean growth rate of tumors developing in animals of the control and fat-enriched groups. These results are not unexpected since it is probable that only dietary changes which drastically affect the health and weight of a tumor-bearing animal will significantly alter the growth rate of its tumor.

Significance.—The same factors which lead to an increased incidence of cancer in mice on a high-fat diet may be responsible for the increased incidence of cancer observed in overweight human subjects (9). It should also be pointed out that these investigations indicate the danger of generalizing from the results of an experimental procedure on only one type of tumor.

SUMMARY

1. By utilizing the spontaneous breast carcinoma, induced skin tumor, induced sarcoma, and primary lung tumor of the mouse, the effects of a high-fat diet on the genesis of tumors were studied.

2. The most striking result of these investigations is the diversity of effects produced by a high-fat diet: (a) The incidence of the spontaneous breast carcinoma was significantly increased. (b) The incidence of the induced skin tumor was increased. (c) The incidence of the primary lung tumor was unaffected. (d) The incidence of the induced sarcoma was unaffected or actually inhibited.

3. A high-fat diet not only produced a definite increase in the incidence of spontaneous breast and induced skin tumors, but also shortened the mean time of appearance of these tumors.

4. The mean growth rate of sarcomas arising in the high-fat group was not significantly different from that of sarcomas arising in the control group.

5. A twofold action of a high-fat diet (solvent action and cocarcinogenic action) is postulated to explain the diverse effects on tumor formation.

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Persistence and Growth of Spontaneous Mammary Tumors and Hyperplastic Nodules in Hypophysectomized Mice*

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The growth of tumors transplanted into hypophysectomized animals usually continues at a decreased rate, but the removal of the hypophysis does not prevent progressive development of the malignant tissue (1, 2, 3, 18). Some investigations indicated that the retarded rate of tumorous growth in hypophysectomized hosts was not attributable to a concomitant general metabolic deficiency (18). The decreased intake of food was not the sole factor responsible for the decreased rate of growth of the tumors. Hypophysectomized mice painted with 3,4-benzpyrene dissolved in benzol developed papillomas and carcinomas although the incidence was lower and the latent period longer than in unoperated controls (15).

With the exception of the interval during the latter part of pregnancy the hypophysis is necessary for the complete proliferation and function of the mammary glands (8, 19). In normal mice the pituitary gland stimulates mammary proliferation either indirectly by regulating ovarian function or directly if the hypophysis is assumed to produce a specific mammatrophic substance under ovarian influence (27).

The mammary glands of mice from tumor-susceptible strains, either from animals treated with estrogens or untreated females, showed localized proliferative areas under certain conditions (7, 9, 11, 13). These nodular areas varied greatly in microscopic and gross appearance. Some nodules were composed of small lobules of alveoli in a secretory or inactive state, abnormal only in that they appeared in otherwise atrophic glands. Other nodules had a more atypical structure consisting of more hyperplastic cells arranged in ducts or alveolar structures, some being diffuse or nodular adenomatous growths. Other small nodules were considered to be adenomas and finally others, small adenocarcinomas. In any one animal all the various morphological types of localized hyperplasias could be found simultaneously. Some of the persisting lobules and localized hyperplastic areas of mammary tissue were capable of regression, as indicated by fibrosis or atrophy with or without infiltration of round cells.

The present investigation was undertaken to determine whether the removal of the hypophysis might alter the structure or incidence of the localized hyperplastic or adenomatous nodules in the mammary gland, and incidentally to determine the effect upon mammary adenocarcinomas in the same animal.

MATERIALS AND METHODS

Forty-seven multiparous first-generation hybrid mice of 4 groups were used in these experiments. The mice were obtained by mating male and female mice of the A and C3H strains, and female mice of the A and C3H strains with males of the C57 strain [1] (Table I). Multiparous female mice of the A and C3H strains show a high incidence of mammary tumors (23, 24). Mammary tumors rarely appear in breeding females of the C57 strain.

The female mice studied were caged with males after weaning. Newborn young were removed following birth in order that pregnancies might follow one another in rapid succession. The onset of pregnancy, when determined, was dated from the morning following the birth of a litter because mice usually mate during the first day following parturition. The pregnant mice were hypophysectomized at from the 9th to the 19th day of gestation, by the operative technic described by Thomas (26). The hypophysectomized pregnant and nonpregnant mice and the intact control mice all had one or more mammary tumors varying from a few mm. to 2 or 3 cm. in diameter at the time they were selected for the experiment (Tables II to IX). Body weights were recorded daily and the tumors were measured at irregular intervals. Only the greatest diameters of the tumors were determined in some mice. In other animals 2 diameters at right angles to each other and more or less parallel to the body wall were obtained.

The mice were killed at varying periods following parturition or hypophysectomy. The mammary glands were removed with the skin and examined first as "whole mounts." Nodular areas of the glands were removed for a more detailed histological examination after sectioning at 8 to 10 micra. The adrenal glands, ovaries, mammary tumors, areas of mammary tissue, and occasionally the uteri and vaginae were sectioned, stained in hematoxylin and eosin, and examined microscopically. Serial sections of the hypophyseal region were studied to check for persisting fragments of hypophyseal tissue.

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[1] The mice of the parental strains were supplied by Dr. L. C. Strong.

Observations

Six of the 25 mice hypophysectomized when considered to be pregnant, failed to give birth to young (Table I). These mice either resorbed their young, aborted, or were not pregnant. The latter probability was slight since the uteri, palpated through the body wall at the time of operation, all contained masses considered to be embryos. Three intact controls likewise failed to give birth to litters.

The 19 hypophysectomized pregnant mice delivered normal litters on the 19th to 21st days after the onset of pregnancy. Delivery usually occurred on the 21st day. Parturition usually was not protracted or abnormal in any way. The young invariably died within 1 to 3 days following birth. The successive replacement with groups of vigorous young in no way stimulated the continuation of milk secretion since, although active nursing was permitted, the young progressively decreased in weight and died.

The Body Weights of Mice Bearing Mammary Tumors

The pregnant mice bearing spontaneous mammary tumors increased in weight until parturition. The increment of body weight of the hypophysectomized mice did not differ appreciably from that of the intact

Table I: Effect of Hypophysectomy on the Progress of Pregnancy and on Parturition

Designation of hybrid mice	Parental strains	Number of mice hypophysectomized	Number pregnant at operation	Number of abortions or failures of pregnancy	Number of controls
A71	A♀ × C57♂	9	7	1	4
AC2	C3H♀ × A♂	9	5	1	3
AC1	A♀ × C3H♂	7	6	4	4
HC2	C3H♀ × C57♂	9	7	0	2
Total		34	25	6	13

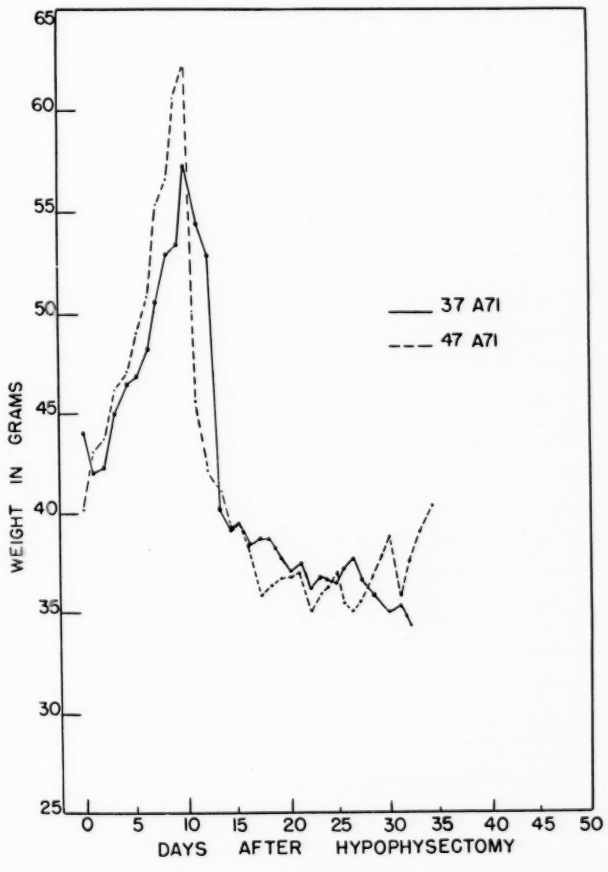


Fig. 1.—Weights of an hypophysectomized (No. 37A71) and a control (No. 47A71) pregnant mouse during the latter half of pregnancy and following parturition.

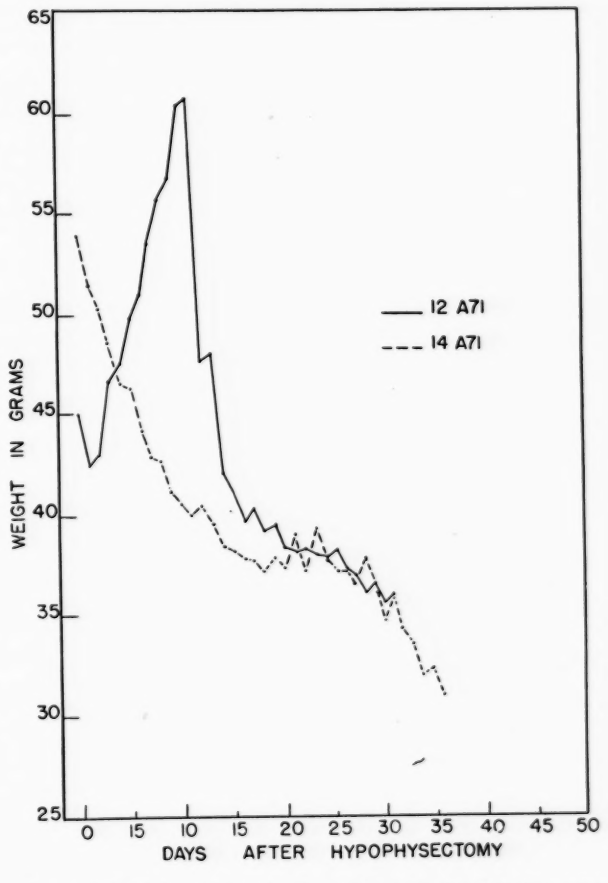
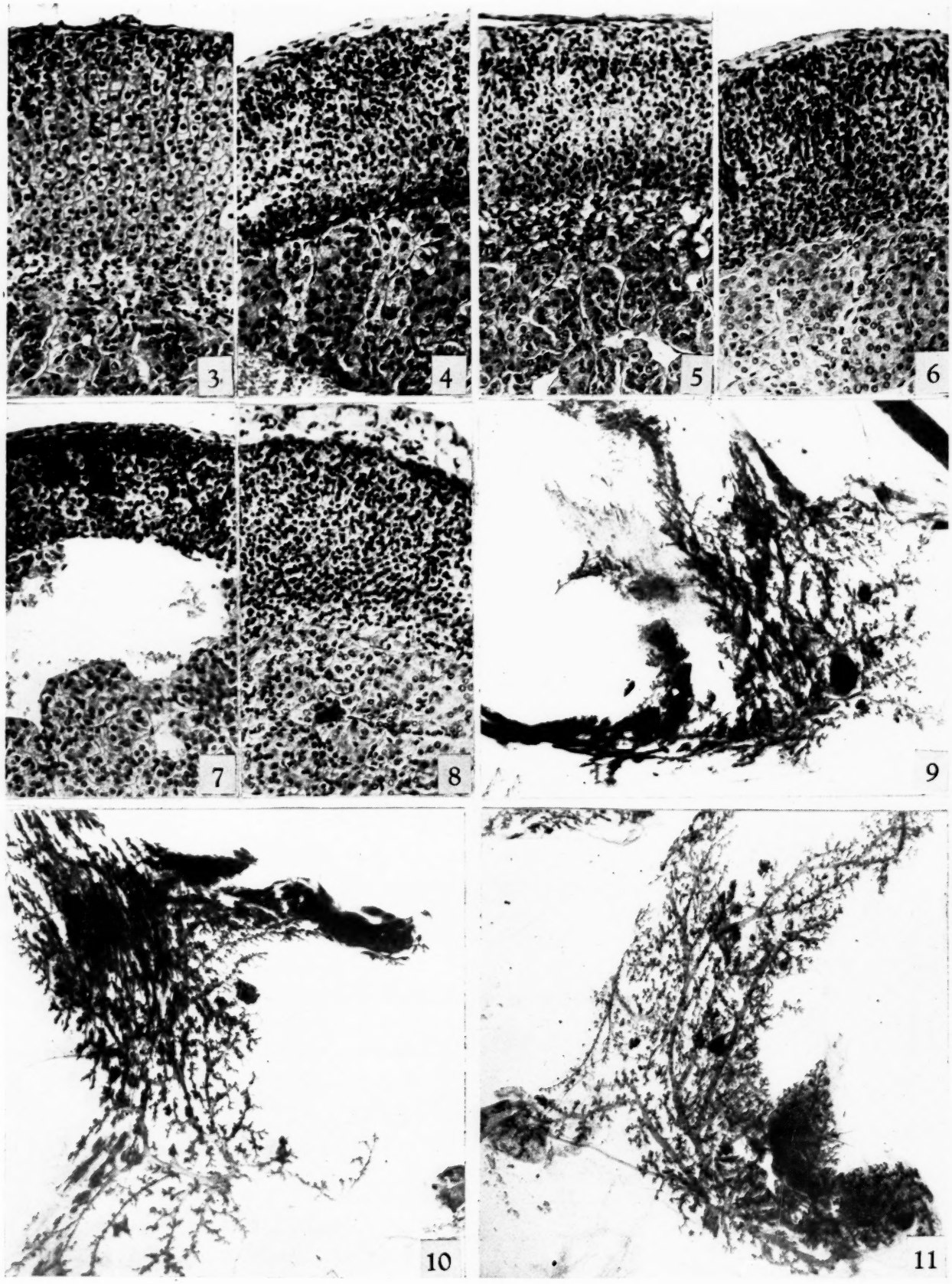


Fig. 2.—Weights of 2 mice hypophysectomized during pregnancy. One mouse (No. 14A71) aborted and the other (No. 12A71) gave birth to a normal litter on the 11th postoperative day.



FIGS. 3–11

pregnant animals until after parturition except during the first day or two following the operation (Figs. 1 and 2). The pregnant uterus and its contents were able to support the typical weight increment of the host in the absence of the hypophysis.

During parturition the total weight decreased precipitously. The body weights of the hypophysectomized mice then decreased at a slower rate until death. While the total weights of the mice decreased, the tumors increased in size in most of the animals. The total weights of the intact mice decreased for 3 to 4 days after parturition and then remained constant or more frequently increased as the tumors increased in size. Daily fluctuations of 5 to 6 gm. in weight occurred in some of these animals.

The mice in which pregnancy was interrupted following operation lost weight progressively, usually more rapidly at first and then at a slower rate.

The mice of the 4 hybrid groups showed no significant differences in the weight changes following hypophysectomy or with the continued growth of the tumor in the intact animal.

Adrenal Glands

The adrenal glands undergo extensive degenerative changes following hypophysectomy (8, 17, 22). The regression of the adrenal cortex occurred even in animals hypophysectomized during pregnancy, although such animals failed to show some other deficiencies associated with the removal of the hypophysis (8).

The adrenal glands of the mice studied in the present series decreased in size following hypophysectomy. The decrease in size involved mainly the adrenal cortex. The fascicular zone decreased in thickness more than the other cortical layers. The zona fasciculata of the intact mice contained large cells with pale cytoplasm and distinct cell membranes (Fig. 3). The cells in the fascicular layer of the hypophysectomized mice were smaller and reduced in number, resulting in an extensive reduction in thickness of this layer (Figs. 4 to 8). The decrease in size of the cortical cells was apparent within a few days after hypophysectomy. The cytoplasm of the cells became increasingly granular and eosinophilic as the duration of the postoperative period was increased. The perimedullary layer of some of the adrenals contained cellular elements undergoing fatty degeneration (Fig. 5). The glomerular zones of the adrenals of some of the hypophysectomized mice were irregularly thickened (Fig. 7). Since this condition of the zona glomerulosa was encountered frequently in the adrenal glands of intact mice it could not be attributed to deficiencies resulting from the operation.

The cortices of the adrenal glands of the control mice of the present series were more irregular in thickness and more variable histologically than the glands of other mice of the same origin. All the control mice had extremely large or multiple tumors whereas the other mice were usually killed when the tumors were smaller. The presence of large tumors may alter the condition of the glands.

The parental strains from which the hybrids were derived showed variable incidences of "brown degeneration" (4). Brownish vacuolated perimedullary cells were found in only one mouse in the present series. The regression of the adrenal glands was similar in the hypophysectomized mice of the different hybrid groups. The adrenal glands of the controls showed no morphological features that could be associated with their different genetic backgrounds.

The Growth of Mammary Adenocarcinomas of Mice of the A71 Group

The mammary cancers of the 9 hypophysectomized and 4 intact mice developed progressively (Tables II

DESCRIPTION OF FIGURES 3 TO 11

Figs. 3 to 8.—Photomicrographs of sections of the adrenal glands of an intact mouse and of mice killed after hypophysectomy. Mag. × 175.

Fig. 3.—From an unoperated control No. 37HC2. The thick fascicular zone contained large cells with a clear cytoplasm.

Fig. 4.—Eighteen days after hypophysectomy (No. 25AC1) the cortex was thin and the fascicular cells were small.

Fig. 5.—Fatty degeneration of the perimedullary zone sometimes appeared as in mouse No. 9AC1 killed on the 21st day after hypophysectomy.

Figs. 6 to 8.—From mice Nos. 35AC1, 22AC1, and 22HC2, and obtained 24, 46, and 56 days after hypophysectomy. The cortices were thin and the cells of the fascicular zones were small.

Figs. 9 to 11.—Photographs of stained and dissected mammary glands.

Fig. 9.—From a mouse (No. 37A71) killed 33 days after operation and 22 days after parturition. One large and 2 smaller nodules are seen in the right central area of the atrophic gland.

Fig. 10.—From a mouse (No. 16A71) hypophysectomized 1 day postpartum and killed 30 days later. One nodular area composed of alveolar and ductal structures is shown near the center of the photograph.

Fig. 11.—From an unoperated control (No. 47A71) killed 29 days after parturition. Several small alveolar nodules are scattered throughout the central part of the gland.

TABLE II: THE GROWTH OF SPONTANEOUS MAMMARY TUMORS IN HYPOPHYSECTOMIZED MICE OF THE A71 STRAIN

Number of animal	Age when tumor appeared, days	Age operated, days	Age killed, days	Time postoperative at death, days	Size of tumor at operation, mm.	Size of tumor at death, mm.	Time operated (dated from parturition), days
28	331	335	339	4	6 × 6	7 × 6	1 pre
38 P	294	297	304	7	5	9 × 12	4 pre
16	294	314	344	30		18 × 18	1 post
					9 × 9	19 × 19	
					22 × 18	27 × 23	
27	278	295	326	31	22 × 17	27 × 25	6 post
12	346	360	392	32	10 × 10	12 × 13	11 pre
37	281	292	325	33		10 × 5	
					16 × 16	21 × 16	11 pre
					16 × 16	25 × 17	
42	279	283	318	35		15 × 10	9 pre
						29 × 16	
					11 × 11	30 × 23	
14	333	337	374	37		6 × 6	
						3 × 2	Aborted
						7 × 5	
					19 × 16	31 × 26	
45	201	249	293	44	17 × 10	18 × 15	7 pre

P = 1 injection of 1 mgm. prolactin on first day postpartum.

TABLE III: THE DEVELOPMENT OF SPONTANEOUS MAMMARY TUMORS IN INTACT MICE OF THE A71 GROUP

Number of mouse	Age when tumor appeared, days	Age tumor measured, days	Age killed, days	Time tumor observed, days	Size of tumor at first measurement, mm.	Size of tumor at death, mm.	Time of first measurement (from parturition), days
33	295	309	322	13	13 × 13	20 × 20	11 pre
					9 × 9	20 × 18	
22	311	351	371	20	34 × 32	32 × 38	1 post
					22 × 20	30 × 26	
47	262	279	302	40	10 × 14	36 × 33	11 pre
34	303	309	358	55		11 × 10	15 pre
						28 × 25	
					6 × 6	32 × 26	

and III). The tumors of the hypophysectomized animals may have grown at a somewhat reduced rate, but it was not greatly reduced. The number of palpable spontaneous mammary adenocarcinomas increased during the period of observation in one of the control and in 4 hypophysectomized mice; and these tumors, first detected after the removal of the pituitary, likewise increased in size.

The cancers of the hypophysectomized mice were histologically comparable to those of the controls and were simple, variable, or papillary cystic adenocarcinomas.[2] Different tumors in the same animal were of different morphological types, and different areas of the same tumor occasionally varied in the arrangements assumed by the tumor cells.

[2] The mammary cancers were classified according to the description given by Cloudman (5).

THE MAMMARY GLANDS OF HYPOPHYSECTOMIZED MICE OF THE A71 GROUP

The mammary glands of the unsuckled lactating mice or of the mice hypophysectomized during pregnancy regressed rapidly, starting immediately following parturition. The rates of regression were not detectably different in the mice of the 2 categories mentioned above. Since the number of mice from which glands were removed during the earlier and more rapid stages of regression was small, slight differences in the rates of mammary regression between the 2 groups would not be detected.

The extent of involution tended to increase with the prolongation of the period following the cessation of suckling and the length of the postoperative interval in the control and hypophysectomized mice respectively. The mammary glands of pregnant hypo-

physectomized mice resembled those of the controls at the time of parturition. After parturition the glands regressed even when young were nursing. A microscopic examination of the gross mounts of the glands of the mice killed more than 7 days after hypophysectomy showed few smaller ducts and no well-formed alveoli. Fewer smaller ducts persisted in the mice killed at later periods (Figs. 9, 10, 11). Cells with a yellowish cytoplasm containing both refractile and stained granules closely surrounded the regressing ducts. These cells were tentatively assumed to be macrophages and to be associated with involution of the glandular tissue.

Nodules of mammary tissue of various sizes were detected in the otherwise atrophic glands of both the hypophysectomized (Figs. 9, 10, 12, 13) and control mice (Fig. 11). Because these localized nodules have been described previously in intact mice of tumor-susceptible strains reference will be limited largely to those in hypophysectomized mice. In the preparations of the dissected glands the nodules varied structurally. Some nodules were small, opaque, densely stained masses, some were composed of lobules of small alveoli or alveoli distended with secretion, or even cystic. Other nodules were composed of diffuse overgrowths of small ducts in either a loose or dense stroma (Figs 12 and 13). Other nodules were composed of cysts and ducts lined by layers of cornified cells and others were obviously undergoing necrosis. Mitotic figures were usually detected in the nodules of all except the 2 latter types. The yellow granular cells associated with the involuted portions of the glands were usually lacking in the nodules showing evidence of proliferation.

Fewer nodules were found in the mammary glands of the hypophysectomized mice than in the controls. An absolute count of the nodules could not be undertaken, since the large tumors present in many of the animals reduced to variable extents the amount of uninvolved mammary tissue available for study from different animals.

A number of nodules were removed from the glands of each animal and sectioned for further microscopic study. Several of the nodules, recognized in the intact glands only as opaque masses, proved to be small tumors histologically indistinguishable from the adenocarcinomas usually found. Other nodules presented histological characteristics described above, as revealed by observation of the stained preparations of the entire glands (Figs. 14, 15, 16, 17). The nodules composed of the masses of small ducts showed hyperplastic epithelial tissue in a moderately cellular stroma (Fig. 14). Some areas contained a preponderance of duct-like epithelial elements. Nodules of this type have been previously described in intact mice as diffuse adenomatous lesions. Small areas histologically similar to the larger adenocarcinomas have been found frequently within these lesions. Another type of nodule composed of ductal structures lined by hyperplastic, irregularly arranged cells was observed in several instances (Figs. 15 and 16). Other nodular mammary areas consisted of ducts or alveolar structures, some of which were lined by a hyperplastic and others by an atrophic epithelium (Fig. 17).

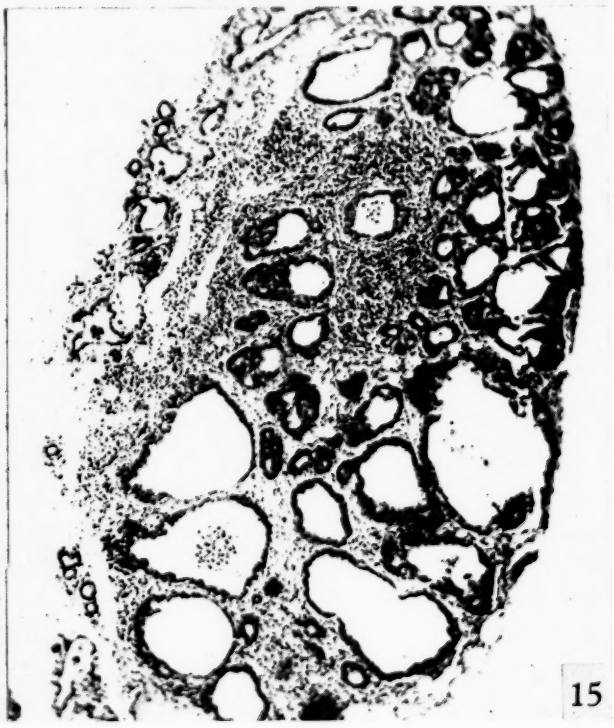
The Mammary Nodules and Adenocarcinomas of Mice of the AC2 Group

The mammary glands of all mice of the AC2 group were removed not less than 7 days after parturition. The glands had all involuted to the extent that only the larger ducts persisted, except in scattered areas occupied by nodules. The glands of 2 mice killed on the 15th postoperative day (Nos. 23AC2, 27AC2, Table IV) contained many small nodules composed of alveolar structures (Fig. 19). Mitotic figures were detected upon microscopic examination of the stained and dissected glands, as well as in several areas removed for histological preparation. Some of the nodules were atrophic (Fig. 20). In these nodules the stroma was infiltrated with round cells and the ducts usually were lined by a stratified, squamous, or even cornified epithelium. The epithelium was largely eroded in some places. Some of these epithelial structures were filled with macrophages. Small areas of the epithelial cells from these lesions showed mitotic figures, indicating some capacity for proliferation. Nodular areas were also found, in the glands of each of the other mice, which resembled in all respects those areas described above or in the mice of the A71 group.

The glands of the hypophysectomized mice contained fewer nodules than those of the unoperated mice. Histologically the nodules of the intact mice ranged from the atrophic lesions described above to small adenocarcinomas.

Progressive growth of the mammary adenocarcinomas occurred in all except 2 of the 9 hypophysectomized animals (Nos. 27AC2 and 37AC2). One of these mice had 2 tumors, one of which increased in size (No. 27AC2). Additional mammary tumors, not observed at the time of hypophysectomy, appeared during the postoperative period in 3 mice and grew progressively until death.

The mammary cancers were of the same morphological types observed in the mice of the A71 group mentioned above. The tumors of the hypophysectomized mice resembled those of the controls.



FIGS. 12 and 13.—Photomicrographs of nodules of mammary tissues from the stained and dissected preparations of the glands.

FIG. 12.—A nodule from a mammary gland of a mouse (No. 27A71) killed 31 days after hypophysectomy. This nodule was composed of a dense network of small hyperplastic ducts indicated at upper margin. Atrophic ducts appear below the nodules. × 95.

FIG. 13.—Same nodule is shown in Fig. 10. This nodule contained small ducts and alveoli. The atrophic mammary tissue is shown at upper left. Mag. × 85.

FIG. 14.—A section through one of the mammary nodules 7 days after hypophysectomy and 3 days postpartum (No. 38A71). The greater part of the nodule consisted of small branching ducts of the type shown in Figs. 12 and 13. A small adenoma is located in the center. Mag. × 50.

FIG. 15.—A section through one of the mammary nodules 32 days after hypophysectomy and 21 days postpartum (12A71). The epithelial elements of this nodule showed mitotic figures and resembled those of larger tumors. The stroma was infiltrated with round cells. Mag. × 90.

The Mammary Nodules and Adenocarcinomas of Mice of the AC1 Group

The mammary glands of the 7 hypophysectomized mice of this group resembled those of the mice of the previous series (Table VI). Nodules of the various types previously described were found in the mammary glands of each animal (Fig. 18). The mammary tumors were, with one exception, simple adenocarcinomas or papillary cystadenocarcinomas.

The cancers of all except one mouse (No. 21AC1) grew progressively following hypophysectomy. In the gross, this tumor differed from all others in this series in that small whitish areas were visible on the external surfaces. Histological examination revealed a tumor composed of large cysts lined by a stratified epithelium. The cysts were filled with sloughed epithelial cells and debris. Few mitotic figures were observed in most areas. Other portions of the tumor resembled an extremely hyperplastic adenocarcinoma. Although this tumor had decreased in size some parts of it were growing. The mammary glands of this mouse which were examined contained several nodules showing squamous epithelium (Fig. 18), and one nodule histologically resembling a small adenocarcinoma. The high incidence of abortion among the mice of this group has been indicated (Table I). The mammary glands therefore probably began to regress at a relatively earlier time after hypophysectomy than if pregnancy had continued.

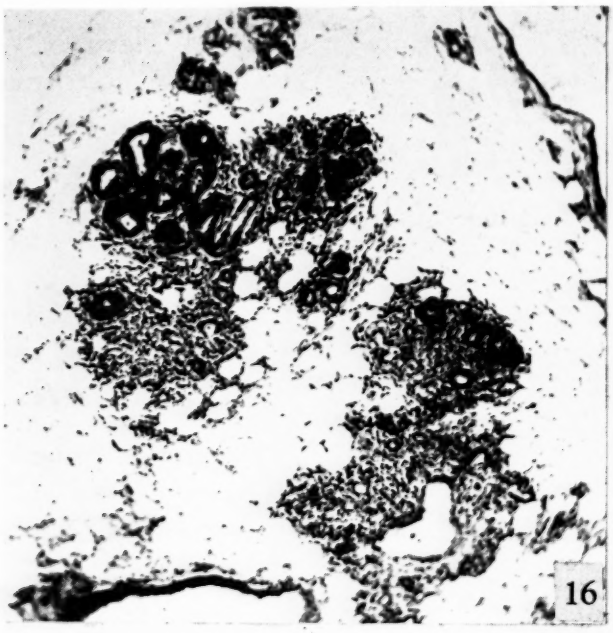
Growth of the mammary tumors of the 4 intact mice continued throughout the period of observation and additional tumors appeared in 1 mouse (Table VII). The tumors were of the same morphological types observed in the hypophysectomized animals.

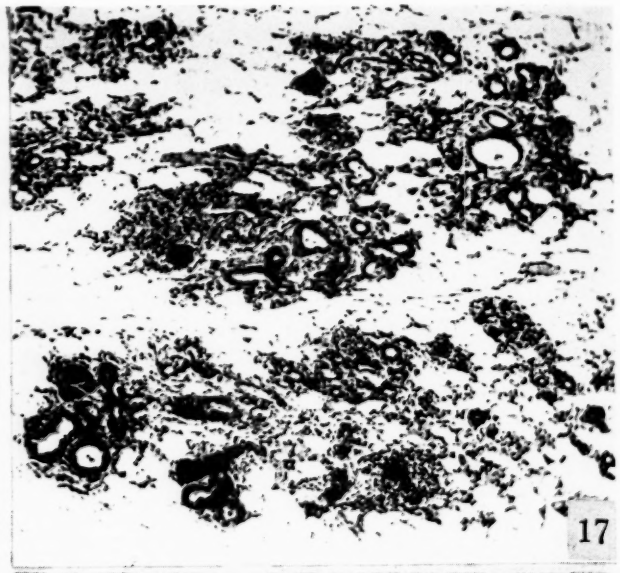
Table IV: The Growth of Spontaneous Mammary Tumors in Hypophysectomized Mice of the AC2 Group

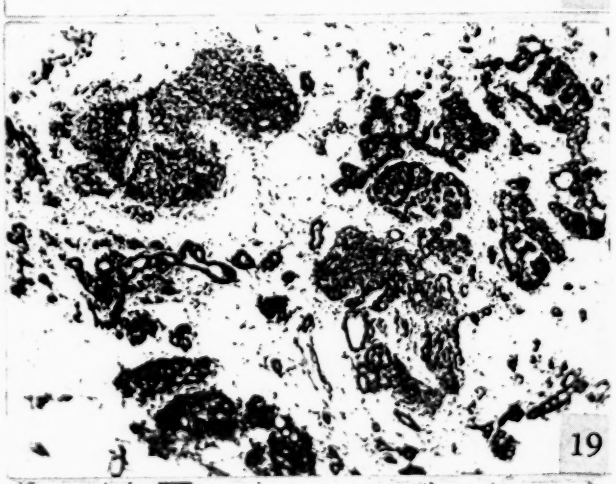
Number of mouse	Age when tumor appeared, days	Age operated, days	Age killed, days	Time postoperative at death, days	Size of tumor at operation, mm.	Size of tumor at death, mm.	Time operated (dated from parturition), days
19	267	291	299	8	17	19	———
23	209	219	229	10	7 × 4	9	———
20	265	287	302	15	14	16	8 pre
					10	12	
					20	24	
27	208	222	238	15	15	19	———
					6	5	
35	207	226	250	24		6 × 6	9 pre
					16 × 15	19 × 19	
					15 × 15	24 × 20	
37	199	226	264	28	19 × 19	11 × 7	10 pre, aborted
50	143	152	181	29	———	20	4 post
					———	16	
40	187	190	223	33		18 × 18	6 pre
					14 × 14	22 × 20	
33	203	214	259	45		12 × 11	11 pre
					10 × 10	17 × 17	

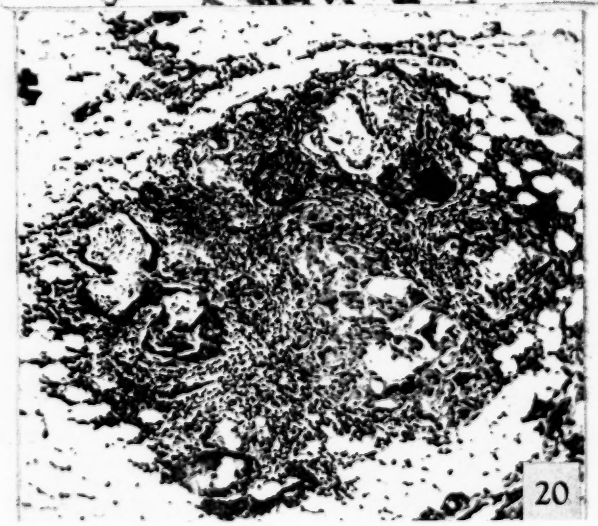
Table V: The Development of Spontaneous Tumors in Intact Mice of the AC2 Group

Number of mouse	Age when tumor appeared, days	Age tumor measured, days	Age killed, days	Time tumor observed, days	Size of tumor at first measurement, mm.	Size of tumor at death, mm.	Time of first measurement (from parturition), days
41	206	238	260	21	19 × 19	24 × 21	26
					30 × 17	35 × 24	
48	184	202	246	44	19	30 × 25	30
					7	10 × 9	
32	204	213	258	45	———	30 × 23	———
					———	30 × 32	









FIGS. 16–20

TABLE VI: THE GROWTH OF SPONTANEOUS MAMMARY TUMORS IN HYPOPHYSECTOMIZED MICE OF THE AC1 GROUP

Number of animal	Age when tumor appeared, days	Age operated, days	Age killed, days	Days postoperative at death	Size of tumor at operation, mm.	Size of tumor at death, mm.	Time operated (dated from parturition), days
36	214	242	245	3	17 × 17	17 × 17	8 pre, aborting
33	274	289	295	6	11 × 11 18 × 15	12 × 13 25 × 24	10 pre
43 P	233	235	242	7	9 × 9	12 × 12 15 × 14	5 pre
21	334	334	344	10	14 × 10	7 × 7	5 pre, aborted
25	313	313	331	18	5 × 5	7 × 4 6 × 6 12 × 10	5 pre, aborted
9	357	371	391	21	18 × 18	23 × 19	8 pre, aborted
22	268	293	339	46	12 × 10	19 × 12	6 post

P = 2 injections of 1 mgm. of prolactin.

TABLE VII: THE DEVELOPMENT OF SPONTANEOUS MAMMARY TUMORS IN INTACT MICE OF THE AC1 GROUP

Number of mouse	Age when tumor appeared, days	Age tumor measured, days	Age killed, days	Time tumor observed, days	Size of tumor at first measurement, mm.	Size of tumor at death, mm.	Time of first measurement (from parturition), days
35	273	273	313	40	7 × 7	33 × 3	4 pre
6	341	365	387	45	34 × 30	48 × 43	8 pre
40	235	235	285	50	12 × 11	11 × 9 10 × 9 21 × 18	13 pre
37	265	265	332	66	7 × 7	35 × 27	11 pre

THE GROWTH OF MAMMARY TUMORS AND NODULES IN MICE OF THE HC2 GROUP

One mammary cancer in 2 of the 9 mice of the HC2 group (Nos. 14 and 25) decreased in size after hypophysectomy although a second tumor appeared and grew progressively in each of these animals (Table VIII). Insufficient data were available to demonstrate whether the rate of growth of the tumors was significantly slower than that of the controls, although such a tendency was indicated (Table IX).

The tumors of both the hypophysectomized and control mice were comparable histologically and in the gross. In both series simple, variable, or papillary cystic adenocarcinomas were present. Mitotic proliferation was evident in the tumors of the hypophysectomized mice as well as in the controls. The impression was obtained that the incidence of mitotic figures was greater in the control than in the hypophysectomized animals.

The condition of the mammary glands again varied

DESCRIPTION OF FIGURES 16 TO 20

FIGS. 16 and 17.—A section through portions of 2 very small nodules from the mouse referred to in Fig. 15. The hyperchromatic structures contained epithelial cells showing active growth. The adjacent mammary tissue was atrophic. Similar nodules appeared in the controls. Mag. × 85.

FIG. 18.—A section through a portion of a mammary nodule from a mouse 21 days after hypophysectomy (No. 9AC1). This nodule, composed chiefly of the small and branching ducts, showed areas of intraductal growth. Mag. × 170.

FIGS. 19 and 20.—Sections of localized nodules of mammary tissue from a mouse killed 10 days after hypophysectomy (No. 23AC2).

FIG. 19.—Areas of hyperplastic cells forming small acini and larger ducts.

FIG. 20.—An atrophic nodule. The stratified epithelium was extensively keratinized and infiltrated with round cells. In some places macrophages filled areas considered to have been formerly lined by a metaplastic stratified epithelium. The dense stroma shows extensive infiltration with round cells. Similar nodules also appeared in unoperated animals.

with the duration of the postparturitional interval. The glands from animals killed 7 or more days after hypophysectomy consisted largely of ducts in a fatty stroma. Small nodules of epithelial tissue were observed in the glands of all except one mouse (No. 25HC2). These nodules resembled those described in animals of the preceding groups. Small fragments of hypophyseal tissue remained in 2 mice. These mice were killed 1 and 7 days after parturition and humoral environment might be assumed when consideration is given to the facts that, when transplanted, such tumors grow progressively in hosts of the same strain which are in extremely different physiological conditions. The transplanted tumors grow in young or old mice, in males or females. In this respect the mammary tumors resemble malignant growths of the connective tissues arising in animals treated with carcinogens, or those of lymphoid tissues (16) or

TABLE VIII: THE GROWTH OF SPONTANEOUS MAMMARY TUMORS IN HYPOPHYSECTOMIZED MICE OF THE HC2 GROUP

Number of mouse	Age when tumor appeared, days	Age operated, days	Age killed, days	Time postoperative at death, days	Size of tumor at operation, mm.	Size of tumor at death, mm.	Time operated (dated from parturition), days
9	261	276	278	2	22 × 21	22 × 21	———
15 IC	307	323	332	9		8 × 6	8 pre
						10 × 8	
					8 × 8	16 × 12	
45	234	237	246	9	3 × 4	5 × 5	4 pre
					10 × 7	15 × 11	
21 P, IC	310	310	324	14		3 × 4	7 pre
						11 × 11	
					5	17 × 11	
20	267	279	309	30	10	14	11 pre
					14	28	
14	258	261	298	37		6	———
					14	8	
47	179	199	239	40		9 × 6	10 pre
					7	34 × 30	
25	239	260	302	42		8 × 6	10 pre
					15	7 × 10	
22	294	294	350	56		6 × 6	9 pre
					6	22 × 18	

IC = Small piece of pituitary tissue remaining.
P = 5 injections of 1 mgm. of prolactin.

TABLE IX: THE DEVELOPMENT OF SPONTANEOUS TUMORS IN INTACT MICE OF THE HC2 GROUP

Number of mouse	Age when tumor appeared, days	Age when tumor measured, days	Age killed, days	Time tumor observed, days	Size of tumor at first measurement, mm.	Size of tumor at death, mm.	Time of first measurement (from parturition), days
37	220	234	246	12		11 × 7	10 pre
					11	21 × 16	
					9	21 × 20	
41	212	228	248	20	29 × 19	44 × 26	10 pre

9 and 14 days after hypophysectomy. The mammary glands of both of these mice showed alveolar structures throughout. The involution of the mammary glands of the mouse killed on the 7th day after parturition apparently had been inhibited by the injections of prolactin.

DISCUSSION

The independence of the growth of mammary adenocarcinomas in mice from any delicately adjusted of the uterine cervix (9). The mammary tumors differ from some tumors of the ovary (25), testis (14), or hypophysis (21), which seem to require rather a special physiological environment for their progressive growth when transplanted into other animals of the same strains.

The continuous growth of malignant transplanted tumors in rats from which the hypophyses had been removed (1, 18) further demonstrates the anabolic

capacity of neoplastic tissue in an environment incapable of supporting progressive growth of the host's tissues. The appearance of tumors in hypophysectomized mice following the application of carcinogens revealed that tumors not only grow in such an environment but that the quality of malignancy can be assumed under such conditions (15).

With very limited exceptions the removal of the hypophysis from rodents results in the establishment of a metabolic equilibrium, in that tissue proliferation merely compensates for tissue destruction. Certain organs for which the hypophysis supplies specific trophic stimulation, such as the gonads, and therefore indirectly the accessory genital organs, and the adrenal glands, undergo marked regression after hypophysectomy (22). The mammary glands are unique in that they regress both following removal or regression of the ovaries, and also may depend on the pituitary for their proliferation as well as for the assumption and maintenance of lactiferous secretion (27).

Localized proliferation of mammary tissue occurred in intact mice from strains susceptible to mammary cancer, while the rest of the glands were regressing or inactive (10, 13). Different areas of the glands therefore showed varying growth responses in a supposedly uniform environment. These nodular areas were atypical in that their constituent cells were capable of proliferation in an environment which adjacent mammary tissue found incompatible. The nodules were limited almost entirely to the glands of mice from strains susceptible to mammary tumors. The number of nodules was greater in estrogen-treated mice than in untreated mice of similar ages (7).

Estrogens had little effect upon the mammary glands of hypophysectomized mice although the injection of some combinations of steroid hormones was followed by mammary growth (6). The hypophysis may produce a specific substance (mammogen) which by itself (27), or a substance such as lactogen which in combination with estrogen, induces mammary growth (12). The present observations reveal that some of the localized mammary nodules persist and proliferate even following ablation of the hypophysis. These nodules, differing morphologically from the adenocarcinomas, resemble the larger tumors in that they have undergone a transformation permitting their survival under conditions inadequate for the tissues from which they arose.

The material available was not adequate for a comparative study of the number of nodules persisting in hypophysectomized and in intact mice. The impression was gained that the number was smaller in the hypophysectomized animals.

The reasons for the use of hybrid mice in this investigation should be emphasized. Animals of the first hybrid generation should be as uniform as the least homogeneous parent strain (20). In addition they show the "hybrid vigor" desirable for such drastic experiments. Experiments, not reported here, on hypophysectomized mice of the C3H strain bearing tumors were much less enlightening because of the shorter average period of survival. The observations on the mammary glands and tumors of the mice of the inbred strain were consistent with those of the hybrids as far as determinable.

A rapid involution of the mammary glands of mice followed hypophysectomy unless the operation was performed during pregnancy. The course of pregnancy was usually unaltered in mice operated on later than the 10th day after mating or during the latter half of gestation. The mammary glands of mice hypophysectomized during pregnancy developed until the time of delivery (8) as did those of control animals. The pregnant uterus and its contents thus supported mammary growth in the absence of the hypophysis. Also the total weights of the pregnant hypophysectomized mice increased in much the same manner as the weights of the unoperated pregnant animals. The fetuses have been removed and a similar mammary proliferation observed, indicating that the fetuses or fetal hypophyses did not contribute to the mammary stimulation (19).

The atrophy of the adrenal cortex following hypophysectomy and the persistent growth of both the mammary nodules and adenocarcinoma indicate the absence of any extensive limitation of tumorous growth by these glands. It cannot be stated, however, that the hypophysectomized mice had a complete adrenal deficiency since adrenalectomy is much more deleterious to continued existence in mice than the removal of the hypophysis.

SUMMARY AND CONCLUSIONS

Thirty-four first-generation hybrid mice bearing spontaneous mammary tumors were hypophysectomized either during the latter half of pregnancy or postpartum. The body weights of most of the pregnant hypophysectomized mice increased to the same extent as those of the pregnant controls. The mice were killed at periods up to 56 days postoperatively. The mammary cancers of most of the hypophysectomized mice grew progressively and new tumors frequently appeared. Localized hyperplastic nodules ("precancerous") were found in the otherwise atrophic mammary glands of the hypophysectomized mice. The adrenal cortices of the hypophysectomized mice decreased in thickness and the cortical cells decreased in size. The mammary glands of the 13 intact control mice may have contained more hyperplastic nodules. The microscopic structure of the tumors and nodules

of the hypophysectomized and intact mice were similar.

The progressive growth of spontaneous mammary adenocarcinomas and of "precancerous" localized hyperplastic nodules occurred in the absence of the hypophysis.

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The Constancy under Varying Conditions of a Transplanted Mammary Carcinoma in Inbred Rats

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The vicissitudes of the transplanted tumor form an interesting commentary on the development of the experimental approach to the study of cancer. For many years propagable tumors constituted the chief means of observing the phenomena of neoplastic proliferation in animals. Despite isolated examples of successful heterologous transfer among innumerable fruitless trials, it was realized that consistent serial transmission of tumors was synonymous with homologous transfer. Many strains of tumors have been thus employed as a means of observing rates of growth of cancer cells under normal and abnormal conditions. Nevertheless, unaccountable variations in the outcome of successive generations of tumor grafts occurred and these proved a fertile source of misinterpretations. The authors of the host of conflicting opinions regarding the effects of any given intervention on the growth of cancer cells, opinions too often based upon observations on limited numbers of animals, had frequently neglected to take into account the uncontrollable variability of their experimental material.

Cancers induced by tar were yet too laboriously obtained to threaten seriously the employment of the transplanted tumor for the daily needs of the investigator. With the isolation and synthesis of the carcinogenic hydrocarbons, however, and the realization of the ease with which they provoke tumors, the opinion became widely held that all experiments with the propagable neoplasm should be abandoned. This radical and unjustifiable view did not prevail, fortunately, because almost concurrently the employment of genetically controlled animal stocks for the transplantation of tumors arising in them was becoming more widely practiced.

Early studies, especially of Loeb (12), Tyzzer and Little (22, 11), and Strong (19) demonstrated the importance of genetic factors in determining the fate of tumor grafts. Little (10) has reviewed the studies on mouse tumors. Observations on rats were reported by Dunning, Curtis, and Bullock (7). In spite of these reports, it has come to be generally appreciated only recently that tumors arising in an animal of a highly inbred strain are transmissible in practically 100 per cent of other members of the same strain or in the F_1 hybrids derived from a cross with an individual of known nonsusceptible alien strain, while those totally unrelated, with an occasional exception, are resistant to grafts. Furthermore, the results in genetically controlled stocks are always reproducible and predictable, and without doubt propagable tumors in such strains now furnish, for many purposes, the next most suitable material to the spontaneous neoplasm. Formerly, without the availability of animals of known lineage, a considerable proportion of the attempts to transplant tumors were unsuccessful, and nontransmissible neoplasms were falsely considered to be endowed with less malignant properties.

In charting the results of consecutive transplant generations of a tumor the pioneer investigators (2) who noted fluctuations in the percentage of successful inoculations thought these related either to seasonal variation, age and sex of the inoculated animals, their diet, or, if these factors appeared inconsequential, dependent upon intrinsic variations in the malignant potentialities of the cancer cells. On the basis of the available data it appeared possible to note rhythms, characterized by a rise and fall in the percentage of successfully inoculated animals. Elaborate mathematical dissertations, as those by Ottensooser (13) and Schreck (17), demonstrating this inherent variability in the growth of commonly used tumors in purchased or nonpedigreed, and necessarily nonhomogeneous stocks, have not been lacking. As the importance of uniformity in animal material was not appreciated then as now, these authors concluded erroneously that an ebb and flow existed in the malignancy of any given tumor strain. Bittner (5) eliminated this hypothesis by demonstrating that with genetically controlled stocks such curves could be reproduced at will if each group of inoculated hosts were constituted of varying percentages of known susceptible and nonsusceptible individuals. In addition Bittner (6), by transplanting 3 different tumors arising spontaneously in an F_1 hybrid, showed that mice of a certain genetic character remained constant in their susceptibilities to each tumor, the percentages of successfully inoculated F_2 hybrids and backcross generations depending precisely on the number of dominant mendelian factors required for the growth of each tumor. Nevertheless, the publication of Symeonidis (21) may be cited as an example of the persisting belief in fluctuation in the virulence of tumor cells, based as always on experiments with uncontrolled animal stocks.

EXPERIMENTAL

The theory of variation in the malignant qualities of a cancer strain was usually accompanied by a second concept: the greater likelihood of successful maintenance of a propagable tumor when the few uncertain transplant generations that followed inoculation of the primary tumor had been bridged. The tumor then would grow in a greater percentage of animals, and more rapidly too, as a process of selection of the most

malignant or the hardiest cells must naturally occur with each transplant generation. The experimental data, obtained with tumors which arose in animals of unknown constitution and were propagated in unrelated individuals likewise of unknown lineage, have at times suggested this possibility. In such a case, in contrast to the ideal conditions of proliferation of tumors maintained in inbred stocks, it might be argued that selection of the most malignant elements would occur when neoplastic cells were subjected to the artificially difficult conditions of proliferation in animals heterozygous for the factors required for their growth. Nevertheless, with the variability of the animal material employed under such circumstances, and the inability of the experimenter to duplicate it, it is manifestly impossible to know whether the animals that proved more susceptible in late transplant generations might not likewise have been so if employed for the earlier series. The counterpart of this thesis is the not uncommon experience of complete failure of transplantation following a number of early, relatively successful generations. In the latter case the result appears again to depend upon the fortuitous use of animals better adapted for a given tumor than others. It is obvious, of course, that the concept of increased virulence of tumor cells on serial passage owes a great deal of its acceptance to an analogy with the findings in bacterial diseases, for in the latter case it is well known that an extracellular agent does increase in virulence when passed serially through appropriate hosts.

The observations here recorded bring conclusive evidence that no change of virulence is evident in a tumor arising in an individual of an inbred strain and propagated within this strain. The tumor (R 2426) employed is a transplantable mammary adenocarcinoma of the rat which has been described in an earlier publication (8). It arose spontaneously in a female of the 27th brother-by-sister generation of the August line. Growth of transplants of this tumor is not influenced by the age or sex of inoculated animals, by pregnancy, or by seasonal variation. The uniformity of animals of this and succeeding brother-by-sister generations employed for grafting is revealed by the consistent success of transplantation. The tumor is now in its 23rd transplant generation, has been propagated for more than 3 years, and has never failed to proliferate progressively in approximately 700 inoculated rats of the strain of the original tumor-bearer.

An ideal characteristic of this tumor for the present study has been its consistently slow rate of growth. If during this time a selection of the more malignant elements had taken place, it would be reasonable to assume that grafts of recent generations would proliferate more rapidly than those of earlier ones. Yet they did not. As Fig. 1 shows, their rate of growth remained constant. Thus, in uniform stocks, it would appear that, barring mutations in tumors, the type of proliferation assumed under the usual conditions of transplantation by any race of cancer cells is fixed permanently at the time the cells become malignant.

There is some variation in the size of tumors in individual animals of each group, but this is caused by the impossibility of measuring exactly the quantity of material used for inoculation. Furthermore, even though it were possible physically to approximate a uniform dose for each inoculum, this would be no guarantee of their physiologic equality. Nevertheless,

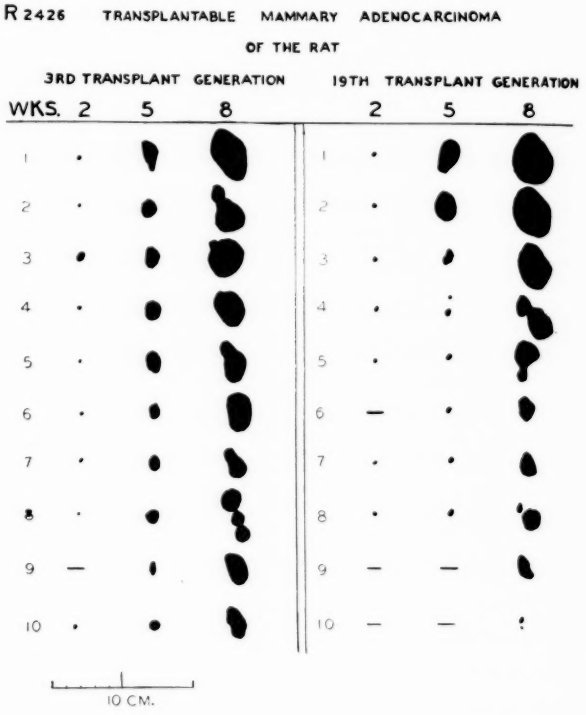

FIG. 1

the variability of one group is comparable with that of another. Records of the first 2 transplant generations are incomplete, but the general appearance of the tumors was not unlike that of tumors charted later. During the period in which observations have been conducted, tumors of some generations attained a large size rapidly, while others proliferated more slowly. This type of variation did not occur with any semblance of regularity, and was totally unrelated to the position of the generation in the series. As stated above, it probably depended upon the varying dimensions or condition of the inoculated fragments.

Animals of some tumor generations received simultaneously 2 grafts, 1 in each groin, or 4 grafts, 1 in each groin and axilla. Bittner (3) earlier reported that quadruple inoculation of a tumor in genetically

controlled but not uniformly susceptible stocks gave rise to tumors either at all sites or at none. Genetic constitution thus determined the fate of transplants. Similarly with rat carcinoma R 2426, 2 or 4 tumors always arose from as many inoculated fragments, and each tumor proliferated independently. The average size of the 2 or 4 tumors in a single host was not different from the dimensions of single tumors in other hosts. Therefore there appears to be no foundation for another old and almost completely discarded theory; namely, that proliferation of a tumor graft is insured by certain specific growth factors in the body existing in limited quantities only. On the contrary, the results of many investigations have demonstrated conclusively that growth of a transplanted tumor is guaranteed by intrinsic genetic factors with which both host and tumor cell are permanently equipped.

Subcutaneous tumor transplants in partially related and totally unrelated rats.—As previously recorded (8), the mammary adenocarcinoma (R 2426) arose in a rat of the August line formed by a cross of individuals of lines 990 and 1561. The persisting genetic relationship of line 990, inbred as a distinct family, to August rats was evident from the considerable percentage of animals of the former strain in which the tumor developed. Of 19 rats of line 990 tested with fragments from the 5th tumor generation 11 (58 per cent) were successfully grafted. An additional 41 individuals were inoculated with fragments from the 15th tumor generation and 32 (78 per cent) were susceptible. The difference in percentages of the 2 series is not significant when consideration is given to the small number of animals employed.

Also reported previously (8) was the fate of the first group of transplants in alien stocks. A proliferating tumor developed in only 1 of 45 inoculated animals from 7 alien inbred strains of rats. These animals bore the 3rd transplant generation. The 16th generation was virtually identical, only 2 carcinomas developing among 93 inoculated animals of alien strains.

From these additional observations on genetically uniform animals it is again apparent that the biologic characteristics of the tumor have remained constant. With continued transplantation there has been no indication of increasing malignancy with correspondingly better growth in alien strains.

It is impossible to explain why an occasional animal from unrelated strains, which on the basis of genetic makeup should be uniformly resistant, does nevertheless prove susceptible. A high degree of homogeneity has been attained in the unrelated stocks, and it is reasonable to assume that all should be either resistant or susceptible. No doubt for practical purposes the susceptibility of alien animals does approach zero, and to account for the rare exception it is necessary to assume that by chance, or for reasons not understood, circumstances favorable to the growth of the graft were encountered when ordinarily they were not expected to occur. While it is apparent that progressive growth of a tumor graft is dependent on genetic factors, the nature of the resistant and receptive states is not entirely clear, though there is evidence to suggest that the former results from a reaction against more or less foreign cells and the latter from the partial or complete absence of such a defense.

As was to have been expected, when a graft of carcinoma R 2426 did give rise to a tumor in partially related or alien animals, its rate of proliferation was considerably less than that of tumors produced by transplants in the August stock of which the original tumor bearer had been a member. In the former case, furthermore, regressions were not uncommon, and healthy tumor was limited to a narrow peripheral zone which surrounded a mass of necrotic material. In contrast was the almost uniformly healthy tumor in the animals genetically adapted for it, except when grafts attained a huge size or ulceration supervened. Growths in animals either incompletely adapted or totally nonadapted resemble, therefore, the common transplantable tumors maintained in unrelated stocks. In the latter instance the enormous mass of necrotic tissue, contrasting so strikingly with a small rim of viable tumor, must in a large measure be a product of the adverse conditions encountered in alien animals.

The possibility of mutations in tumor strains maintained in genetically controlled stocks has been demonstrated. Bittner (4) stated that during propagation of 2 spontaneous tumors from 1 animal the percentage of susceptible individuals in the F_2 hybrids increased and the tumors grew more vigorously after a certain number of transfer generations. Thus it was surmised that the presence in hosts of a smaller number of dominant mendelian factors was then required for progressive growth of the tumors, and it is possible to interpret this as a sign of greater malignancy. In the experiment of Strong (18), however, the mutation resulted in a complete loss of genetic specificity by the tumor, for in addition to more rapid growth of grafts, mice of alien strains, known previously to be resistant, now proved susceptible.

Furthermore, Lewis and Lichtenstein (9) reported that induced sarcomas from one inbred strain, repeatedly inoculated into animals of another, eventually nullified the resistance of the latter and allowed proliferation of the grafts. Somewhat similar results were reported earlier with tumors that arose and were maintained in animals of unknown lineage, as for example by Raposo (15), who stated that repeated

inoculation of carcinoma 63 may give rise to tumors in originally resistant mice.

Intravenous inoculation of tumor in partially related and totally unrelated rats.—Tumor emulsions injected into the tail vein produced, in all August rats, extraordinarily diffuse pulmonary tumors varying in size from those of microscopic proportions to nodules several centimeters in diameter (8). It may be assumed that the injected material was thoroughly distributed in the pulmonary fields, and that each tumor nidus arose independently from small numbers or groups of cells. When compared with subcutaneous sites, where the proliferation of small quantities of tumor proceeds slowly, this would appear to indicate a favorable terrain in the lungs for the multiplication of neoplastic cells, especially since histologic examination of the lung nodules disclosed a minimum or total absence of reactive changes in the surrounding pulmonary tissue. Additional strength is lent to this supposition by the later observation of Andervont and Shimkin (1), who reported that intravenous injection of an adenomatous gastric lesion of mice resulted in pulmonary growths, while subcutaneous transplantation was unsuccessful.

Emulsions of the rat carcinoma were injected simultaneously into the tail vein and the subcutaneous tissue of the partially related animals of line 990 and into alien rats to ascertain whether the lungs do constitute a favorable site for the growth of a tumor, which, because of genetic differences of the inoculated animals from the host in which it arose, proliferates subcutaneously only with difficulty. Of 18 rats of line 990 tumors grew at both sites in 7, only subcutaneously in 5, in the lungs alone in 2, while 4 were negative at both sites. The results in this small group indicate a random occurrence of a positive or negative outcome at either one or both sites. Of 21 animals of totally alien strains 1 each had pulmonary or subcutaneous tumor, and 1 was positive at both locations. The growths in the lungs in both groups did not have the widespread diffusion of those in August rats, but were in the form of a few scattered small nodules or a single larger mass. Thus the lungs do not appear to constitute invariably a more favorable site than the subcutis for the growth of a tumor arising in an unrelated animal.

Transplantation of tumors originating from irradiated fragments.—As has been recorded (8) it is necessary to administer approximately 5,500 r *in vitro* to small fragments of the tumor to abolish its proliferative capacity. This dose is somewhat higher than is generally reported to be required for the destruction of the common transplantable tumors maintained in animals of unknown and variable genetic constitution and in all probability unrelated to the original tumor-bearer. In the latter case the adverse conditions under which the tumor must proliferate in animals only incompletely adapted for it must contribute to an artificial decrease in its resistance to radiation.

Thus Sugiura (20) stated that a dose of 2,800 to 3,000 r *in vitro* is lethal to sarcoma 180 of the mouse, while the dose required *in vivo* appeared to be considerably below this amount. Oughterson, Tennant, and Lawrence (14), however, showed the determining influence of genetic constitution of the host on the radiosensitivity of tumors. To produce regressions of a propagable mammary cancer, which arose spontaneously in a mouse of the highly inbred A strain and was transplantable in 100 per cent of the animals of this strain, a dose of 5,000 r *in vivo* was required. Regressions occurred spontaneously in 28 per cent of F_2 hybrids of a cross with C57 black mice, and tumors in 82 per cent of the hybrids were cured by 2,500 r.

In the present experiments, when doses of 4,000 to 5,000 r were given *in vitro* to fragments of the mammary cancer (R 2426) of the rat, some of the transplanted fragments grew. Their latent period was considerably prolonged and the slow growth of any ensuing tumors indicated an origin in a small number of cells or in damaged elements. Histologically such tumors did not, however, differ from those arising from untreated fragments.

Tumors in August rats which were produced by fragments that had received 4,000, 4,500, and 5,000 r respectively were grafted into both groins and the right axilla of 9 additional August rats and of 20 animals of alien strains. All fragments in August rats produced tumors which in growth rate and structure did not differ from those produced by routine implants, while none of the grafts in unrelated animals showed evidence of proliferation.

Under the conditions of the experiment, no evidence existed, therefore, of the production by roentgen radiation of a mutation in the tumor. Reinhard, Warner, and Goltz (16), on the other hand, asserted that a mutation in a transplantable mammary cancer of an inbred strain of mice occurred when a dose of 50 to 100 r was administered to the tumor. Following this quantity of radiation it was possible to inoculate the tumor in series in a considerable percentage of alien animals not susceptible to normal grafts.

SUMMARY

The proliferative rate of grafts from early and recent generations of a transplantable mammary adenocarcinoma (R 2426) of the rat has been compared. The morphology of this neoplasm, now in its 23rd transplant generation, has remained constant and tumors continue to be remarkably free of the extensive necrotic changes characterizing the common

transplantable neoplasm maintained in stocks of unknown constitution unrelated to the original tumor bearer.

The constant and regular growth of a neoplasm arising in an individual of an inbred strain and maintained in animals of the same strain is illustrated by the similar rate of proliferation of transplants from earliest and latest generations of such a tumor. This transplantable adenocarcinoma appeared from its inception characterized by a slow rate of growth, and the most recently inoculated grafts do not proliferate more rapidly than those of the earliest generations. Thus, if it may be assumed that all tumors are biologically comparable, the rate of growth of tumor cells, under normal conditions, in homozygous hosts appears to be fixed permanently at the time the malignant change occurs. A possible exception would be the development of mutations.

Additional proof for the constant qualities of the neoplastic cell is the equal degree of success in animals of a partially related strain, and the practically uniform failure of grafts from early and late generations of the tumor in alien animals.

Diffuse growth of the tumor occurred in the lungs of August rats injected intravenously with a tumor emulsion, but the pulmonary tissue of partially related and alien animals was not more susceptible than subcutaneous sites.

Grafts of tumors originating in fragments that received 4,000, 4,500, and 5,000 r radiation *in vitro* grew progressively in all August rats, but in no animals of alien strains. Under the conditions of the experiment these doses did not, therefore, induce a mutation in the tumor.

The constant and predictable growth of a transplanted tumor which originates in a member of an inbred strain and is propagated in others of the same strain makes it a suitable, efficient, and dependable medium, obtainable in unlimited quantities, for observations on the proliferation of malignant cells, and hence for an assay of the effect of any treatment instituted by the investigator.

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The Isotopic Constitution of Potassium in Normal Tissue and Cancer from Human Subjects

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In a previous paper (4) results were described of investigations on the isotopic constitution of potassium contained in Jensen rat sarcoma and mouse sarcoma 37, and the relation of these results to those of similar investigations concerning potassium in a series of normal rat tissues (3) was considered. It was found that the isotopic ratio K^{39}/K^{41} of potassium in both kinds of tumors showed, if compared with that of mineral potassium, a slight but definite increase, thus indicating a corresponding decrease in the percentage of the heavy isotope K^{41}. Since a deviation of this nature could not be observed with potassium in any of the normal tissues, it appeared that the isotopic constitution of potassium in normal tissue and tumors was appreciably different. In addition, it was found that potassium in muscle from tumor-bearing animals, contrary to potassium in muscle from normal ones, showed a deviation similar to that observed with potassium in tumors.

The results mentioned above suggested the desirability of comparative investigations on the isotopic constitution of potassium present in normal tissue and cancer from the human subject. Such investigations have been carried out, and their results are described in the present paper.

METHODS

The tissues were taken from postmortem material, in most cases 24 hours after death. Different organs, which on macroscopical examination showed little or no pathological changes, were used as sources of normal tissue.[1] Cancerous tissue was obtained from a number of carcinomas situated in different organs.

Each sample of cancer, after having been freed as far as possible from residues of normal tissue, necrosis, etc., was cut into a number of small pieces; these were rapidly rinsed in distilled water and dried on Petri dishes at about 100° C. The samples of normal tissue were treated in a similar way. The dry material was ashed in a platinum dish, and the ash was directly used for the mass spectrographic measurement as previously described (2,3).

TABLE I: ANALYSIS OF POTASSIUM IN HUMAN CANCER

Sample number	Sex and age	Carcinoma of:	Isotopic ratio K^{39}/K^{41}	Percentage of K^{41}
1	♂, 29 years	Liver (primary)	14.35	6.515
2	♂, 33 years	Lung	14.38	6.502
3		Liver (metastasis)	14.36	6.510
4	♂, 40 years	Stomach	14.35	6.515
5	♂, 63 years	Lung	14.34	6.519
6	♀, 20 years	Rectum	14.38	6.502
7		Liver (metastasis)	14.31	6.532
8	♀, 48 years	Kidney	14.33	6.523
9	♀, 50 years	Lung	14.35	6.515
10	♀, 54 years	Colon	14.34	6.519
11		Liver (metastasis)	14.32	6.527

RESULTS

Table I shows the results with cancer. Of the 11 samples tested 3 were prepared from primary carcinomas of the lung; 1 each from primary carcinomas of the liver, kidney, stomach, colon, rectum; and 3 from metastases of the liver. It will be seen that the isotopic ratio of potassium showed in each case a value higher than 14.20; *i.e.*, the isotopic ratio of mineral potassium (as contained in ordinary potassium chloride, A.R.). The content of the heavy isotope K^{41} was thus decreased. As to the samples from primary carcinomas, this decrease varied between 0.9 and 1.2

[1] Although the tissue thus obtained cannot be regarded as normal in a strict sense, we should like to retain this designation, considering that "normal" means in this connection "non-cancerous."

per cent, the average being 1.0 per cent. In 2 of the samples from liver metastases the isotopic ratio was only slightly less than that in the corresponding primary carcinomas, while in the 3rd sample the reduction was greater. On an average, the K^{41} content of potassium in liver metastases was by 0.9 per cent smaller than that of mineral potassium.

The results with normal tissue are given in Table II. Of the 20 samples tested 4 were prepared from liver, and 3 from kidney (cortex). One liver sample was obtained from a patient with primary liver carcinoma; parts of the organ that appeared normal were taken. The isotopic ratio was slightly greater than that of mineral potassium, the corresponding decrease in the content of K^{41} being 0.5 per cent. A deviation smaller than this (0.3 per cent decrease in K^{41}) was found in 2 other samples, from a patient with tuberculosis and from one with carcinoma of the colon. The remaining sample, obtained from a patient with bronchopneumonia and nephritis, showed a normal isotopic ratio. One kidney sample originated from the subject with carcinoma of the colon. A slight increase of the isotopic ratio was found, the corresponding decrease in the content of K^{41} being 0.6 per cent. The 2 other samples, one from a patient with heart disease and the other from one with lymphatic leukemia, showed no noticeable deviation. Two further samples were prepared from heart muscle; both showed a slight increase of the isotopic ratio (decrease in K^{41} by 0.5 per cent), although in 1 the parallel sample from liver gave a normal value. A number of samples from other organs were tested: 2 from lung, 2 from spleen, 1 from skeletal muscle, and 1 from brain (gray and white matter). None of these samples showed a noticeable deviation from the isotopic ratio of mineral potassium. Lastly, tests were made on 5 samples of bone marrow, obtained from lumbar vertebrae; a certain amount of bone substance (spongiosa) was present. Three of these samples, from patients with heart disease, with cancer, and with diabetes, showed an appreciable decrease of the isotopic ratio. The corresponding increase in the content of K^{41} was, on an average, 1.8 per cent. In the 2 other samples the deviation was also clearly indicated, but less pronounced, the average increase in

TABLE II: ANALYSIS OF POTASSIUM IN HUMAN NORMAL TISSUE

Sample number	Sex and age	Disease	Tissue	Isotopic ratio K^{39}/K^{41}	Percentage of K^{41}
1	♂, 18 years	Calcified pericardium, pleurisy	Kidney	14.20	6.579
2			Spleen	14.20	6.579
3	♂, 29 years	Cancer of liver *	Liver	14.28	6.545
4	♂, 44 years	Endocarditis, pericarditis	Bone marrow	13.90	6.711
5	♂, 56 years	Peritonitis	Lung	14.20	6.579
6	♂, 63 years	Lymphatic leukemia	Kidney	14.22	6.570
7			Lung	14.20	6.579
8			Bone marrow	14.02	6.658
9	♂, 71 years	Chronic tuberculosis of lungs	Liver	14.25	6.557
10		Acute general tuberculosis	Heart muscle	14.27	6.549
11	♂, 73 years	Arteriosclerosis	Skeletal muscle	14.20	6.579
12			Bone marrow	14.06	6.640
13	♀, 17 years	Chronic tuberculosis of lungs Acute general tuberculosis	Brain	14.23	6.566
14	♀, 54 years	Cancer of colon †	Liver	14.25	6.557
15			Kidney	14.29	6.540
16			Bone marrow	13.93	6.698
17	♀, 56 years	Bronchopneumonia, nephritis	Liver	14.20	6.579
18			Heart muscle	14.28	6.545
19			Spleen	14.20	6.579
20	♀, 62 years	Diabetes	Bone marrow	13.96	6.685

* *Cf.* sample 1, Table I.
† *Cf.* samples 10 and 11, Table I.

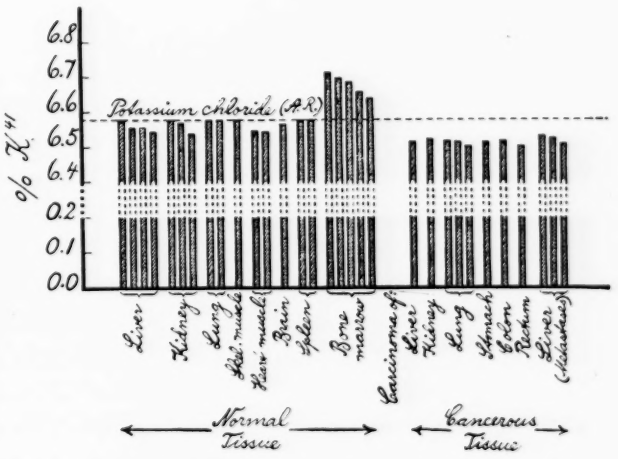

FIG. 1.—Content of K^{41} in potassium present in normal tissue and cancer from human subjects.

K^{41} being 1.1 per cent. The latter samples originated from cases of arteriosclerosis and lymphatic leukemia.

The results obtained with both normal tissue and cancer are combined in Fig. 1. The height of each column indicates the percentage of K^{41} in potassium contained in one ash sample, while the dotted line indicates the percentage of K^{41} in mineral potassium. The columns are interrupted between 0.2 and 6.4 per cent K^{41}.

DISCUSSION

The isotopic ratio of potassium in human cancers, in primary carcinomas as well as liver metastases, has thus shown a slight but definite increase as compared with that of mineral potassium. The deviation is fairly similar to that previously obtained with potassium in animal tumors (4). On the other hand, it is evident that the distinct decrease of the isotopic ratio shown by potassium in human bone marrow agrees approximately with corresponding findings on bone marrow of the rat (3) and other animals (1).

The results with various other normal tissues from the human subject show that the isotopic ratio in 9 samples was very close to that of mineral potassium, while in 6 samples a minute increase was observed. The latter samples included 3 (2 from liver and 1 from kidney) which originated from persons with cancer; this might indicate that potassium in normal tissues from cancer-bearing patients approaches potassium in cancerous tissue in its isotopic constitution. This conclusion is consistent with the result of previous investigations on potassium in muscle from normal and tumor-bearing rats and mice (4); some uncertainty exists, however, owing to the increased values shown by the other 3 samples (2 from heart muscle and 1 from liver), obtained from noncancerous persons. These are the only values which may be regarded as inconsistent with our findings on potassium in normal tissues of the rat; but in making this comparison it is necessary to consider the possible influence of postmortem changes, the chance that other diseases (*e.g.,* tuberculosis) may induce a similar isotope effect and, finally, that even potassium in normal rat tissues (*e.g.,* liver) has shown occasionally a slight increase of its isotopic ratio. Thus it can be said that, in general, the results with normal human tissue agree with those obtained with normal animal tissue.

Considered as a whole, the results indicate an appreciable difference in the isotopic ratios of potassium in normal tissue and cancer from human subjects. In each of the cancer samples tested the isotopic ratio was higher than in any of the samples from normal tissue; and the percentage of the heavy isotope was consequently lower. As regards individual tissues studied in the normal as well as the cancerous state, the difference is most clearly seen when the isotopic ratios obtained with normal lung tissue and lung carcinoma are compared. The difference agrees, both in direction and magnitude, with that shown by potassium in normal tissues and tumors from animals; this similarity is especially striking in view of the different nature of the two kinds of growths: spontaneous carcinoma on the one hand and transmissible sarcoma on the other. The origin of this deviation in the isotopic constitution of cancer potassium, and its possible importance for the cancer problem in general, will be a matter for further investigations.

SUMMARY

The isotopic constitution of potassium in human cancer has been studied and compared with that of potassium in various normal human tissues. An appreciable difference in the isotopic ratio K^{39}/K^{41}, and consequently in the percentage of the heavy isotope K^{41}, was observed. The difference agreed, both in direction and magnitude, with that previously obtained with potassium in normal tissues and tumors from animals.

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Acid and Alkaline Glycerophosphatase in Tissue and Serum*

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Since the publication by Kay (9) of a method for the determination of the glycerophosphatase of blood serum, increasing interest has been manifested in the occurrence of phosphatases in the blood and in various organs in health and disease. It was early established that the phosphatase of bone has its maximum activity in alkaline solution (17), and that that of the prostate gland is active only in acid solution (10, 11). Some confusion existed for a time regarding the pH of maximum activity of the phosphatases of various other tissues. The reason for this became apparent when several independent investigators showed that the liver and kidney contain both acid and alkaline phosphatases which can be separated (1). The Gutmans and their coworkers have shown clearly that bones which are the site of metastases from carcinoma of the prostate contain large amounts of acid phenylphosphatase and alkaline glycerophosphatase (4–8). They have also established the clinical significance of the occurrence of these enzymes in the blood. The great diversity of methods employed in many phosphatase studies has, however, rendered difficult or impossible any quantitative comparison of the results from different laboratories. Hence it seemed desirable to determine the activities of phosphatases from various sources on a single substrate, sodium β-glycerophosphate, over the entire pH range from 3 to 10. The results obtained are the subject of the present paper.

METHOD

The method employed for alkaline serum phosphatase is essentially that of Bodansky (2). This consists in brief in adding 1 volume of serum to 9 volumes of a water solution containing 0.5 per cent sodium β-glycerophosphate and 0.42 per cent monosodium diethylbarbiturate. The mixture is incubated for 1 hour at 37° C., the enzyme is then inactivated by the addition of an equal volume of 10 per cent trichloracetic acid, and the mixture is filtered. At the same time 1 volume of serum is added to 9 volumes of 5 per cent trichloracetic acid in another container, and filtered. The inorganic phosphate in the 2 filtrates is then determined by adding to each 5 cc. of filtrate 4 cc. of a solution of sodium molybdate in sulfuric acid, followed by 1 cc. of a solution of stannous chloride in hydrochloric acid, and comparing with a standard in a colorimeter. The amounts of phosphorus in the filtrates are calculated to those which would have been obtained from 100 cc. of serum under like conditions, and the amount in the unincubated specimen is subtracted from that in the incubated specimen. The difference represents the amount of substrate decomposed by phosphatase. One unit of phosphatase is defined as a phosphatase activity which will liberate 1 mg. of phosphorus as phosphate ion from the given substrate during 1 hour incubation at 37° C. under the experimental conditions described. Substrate is present in large excess at all times.

Our method for tissue phosphatase was based originally on that of Franseen (3) but has been modified so that the results may be more directly comparable with those for serum. One to 5 gm. portions of the fresh tissue are minced finely with scissors or morcellated with rongeurs and weighed (into a flask). Twenty cc. of water per gm. of tissue are then added together with a few drops of toluene, and the mixture is kept in the icebox with occasional shaking for 48 to 72 hours. It is then filtered through washed gauze and the filtrate used for phosphatase determination.

A few modifications of the method for serum phosphatase are necessary in handling tissue extracts owing to the wide range of phosphatase activities and inorganic phosphorus concentrations encountered in the latter. Usually a trial run is necessary before the best conditions can be found. For inorganic phosphate determinations a mixture of 3 cc. of filtrate with 12 cc. of 5 per cent trichloracetic acid often gives a convenient phosphate concentration, but proportions of 0.5:14.5 or 7.5:7.5 must sometimes be used. In the last case 10 per cent trichloracetic acid instead of 5 per cent must be used so as to avoid too low a final acidity. As the phosphatase activities to be measured range from 0.05 units to 500 units per gm. of tissue, the times of incubation must be varied inversely. Incubation times from 2.5 minutes to 24 hours may be used. The relation of time of incubation to amount of phosphate liberated is discussed in detail below.

We have introduced several modifications in the method as originally developed (2). In a previous publication (14) we described a procedure for correcting for reagent blanks. This renders the method somewhat more flexible and assures that contamina-

* This investigation was aided by a grant from The Anna Fuller Fund.

tion of reagents or deterioration of substrate will be discovered. We have found, however, that the correction curves as originally published are not of general validity, but must be redetermined for each lot of reagents. With this technic, satisfactory readings may be obtained when the concentration of the unknown is between one-third and twice the concentration of the standard. When the phosphatase activity is such that the concentration of the unknown is more than twice that of the standard after 1 hour incubation, a shorter incubation is desirable. Readings not more than 15 per cent in error may usually be obtained on unknowns with as high as 5 times the concentration of the standard, however, by taking only 1 or 2 cc. of filtrate and making up to 5 cc. with water. In this case, since the phosphatase correction factor represents the inhibitory effect of the substrate on color development, and since smaller quantities of substrate are present, only one-fifth or two-fifths of the usual correction is made. When the concentration of the unknown is more than 5 times that of the standard, the determination indicates order of magnitude only and must be repeated with appropriate reduction in incubation time.

Some standard makes of filter paper contain significant amounts of phosphate and must not be used. We have always found Whatman No. 1 to be free of contamination and to retain precipitate satisfactorily. Turbid filtrates are occasionally encountered with blood from jaundiced patients or with certain tissue extracts, but these can be cleared by passing a second time through the same filter paper.

Adjustment of pH

Bodansky's substrate, when mixed with serum in the proportions of 10:1, gave a colorimetric pH of 8.6. We early encountered difficulty in preventing absorption of carbon dioxide by the substrate with consequent decrease in pH. We therefore adopted the practice of withdrawing 1 cc. from each serum-substrate or tissue extract-substrate mixture for pH determination. Originally readings for alkaline phosphatase were made in the bicolorimeter with thymol blue; readings for acid phosphatase were made in the comparator with propyl red or bromothymol blue. For most of the work to be reported in the present paper, pH determinations were made with the Beckman pH meter. Fair checks were obtained between readings in the acid range made by the two methods. For alkaline serum-substrate mixtures, however, a serious protein error was found to be present in the colorimetric pH readings, a colorimetric reading of 8.6 being equivalent to an electrometric reading of 9.1. We have, therefore, adopted 9.10 as the pH of reference for alkaline phosphatase determinations.

In practice, alkaline serum phosphatases are usually determined at approximately pH 9.0 and 9.2, and the value at pH 9.1 found by interpolation. Alkaline phosphatase of tissue must always be determined in this way because, as will be explained later, the pH curves of different tissue phosphatases differ enormously. The phosphatase pH curve for serum between pH 8.9 and 9.4, on the other hand, is nearly constant over a wide range of phosphatase activities. For this reason, when it is inconvenient to make duplicate determinations of alkaline serum phosphatase, or when, owing to shift in the substrate alkalinity or to unusually high or low protein content of the serum, the pH values of both serum-substrate mixtures lie on the same side of pH 9.1, the phosphatase readings at the experimental pH may be calculated to those at pH 9.1 by means of the correction curve in Fig. 1. In this curve the phosphatase activity at pH 9.10 is assumed to be unity. The pH values at which the determinations are made are plotted as abscissas against the factors necessary to correct the activities to pH 9.10 as ordinates. The curve was constructed from the results of 165 determinations made on 51 sera whose phosphatase activities ranged from 1.4 to 38.5 units per 100 cc. The deviations of the different points from the curve were not greater than the maximum error of the colorimetric readings. About 500 additional duplicate serum phosphatase readings have been corrected to pH 9.10 by means of the curve with satisfactory results. Inspection of the curve shows that, in the neighborhood of pH 9.10, a shift of 0.10 pH causes a change of about 14 per cent in serum phosphatase activity. The necessity of careful pH control is at once apparent.

Phosphatase determinations in the pH range between 8.0 and 10.5 are made in the usual buffered substrate to which appropriate small amounts of hydrochloric acid or sodium hydroxide have been added. Phosphatase determinations at a pH below 8.0 are made in a solution containing 0.5 per cent sodium glycerophosphate and no buffer. The pH is adjusted to the desired value by addition of hydrochloric acid. The buffering actions of different tissue extracts differ considerably. The buffering action of serum is also somewhat variable, although averaging higher than that of tissue extracts. Hence, the exact amount of acid or alkali which must be added to any given mixture of tissue extract or serum with substrate must be determined by trial. A rough guide to final acid and alkali concentrations which may be expected to give certain pH readings to mixtures of 9 parts of

substrate with 1 part of serum or tissue extract is shown below.

Unbuffered substrate		pH
0.013	HCl	3
0.0125	"	4
0.0115	"	5
0.0080	"	6
0.0030	"	7
0.0	"	8
Buffered substrate		
0.0015	HCl	8.5
0.0	"	9.0
0.0025	NaOH	10.0

Spontaneous acid hydrolysis of the substrate is negligible under the experimental conditions. Samples of unbuffered substrate which had been brought to pH values of 2.3 to 6.4 and incubated for 45 hours at 37° C. failed to show free phosphate ion in concentrations which could be demonstrated with certainty by the colorimetric method. Unbuffered substrate containing a drop of toluene keeps for a month or more in the icebox at its natural pH of about 8.4. At pH values of 6.0 or less it readily becomes moldy with rapid liberation of phosphate ion. Hence, we do not add acid to the stock supply of substrate even when numerous determinations are to be run at the same pH, but adjust the pH just before the solution is to be used.

When solutions containing phosphatase are mixed with substrate and incubated, the pH of the mixture may change. When the incubation is for 1 hour or less, the change is usually small. In a previous publication (13) we stated that, in alkaline serum phosphatase determinations, the average change in the colorimetric pH of the serum substrate mixture during incubation was −0.09 pH. Recently, using the pH meter, we have found the average in 21 alkaline phosphatase determinations to be only −0.01 pH or less than the experimental error. We conclude that the changes previously observed in colorimetric pH may have been due to changes in the color of the solutions. For determinations on acid serum phosphatase incubated 1 to 4 hours, there is often a slight rise in pH, but as the phosphatase activity is nearly constant over a considerable range of acidity, this may be disregarded. In tissue phosphatase determinations where long incubations are necessary the change in pH may be significant. Thus, for 20 alkaline tissue phosphatases incubated 17 to 23 hours, the average change was −0.12 pH and the range was −0.39 pH to +0.06 pH. For 33 acid tissue phosphatases the average change was −0.05 pH and the range was −0.32 pH to +0.23 pH. With long periods of incubation it is therefore desirable to read the pH at both the beginning and the end of the experiment and use the average.

Time of Incubation

Bodansky (2) published correction factors to be applied when times of incubation longer or shorter than 1 hour are employed. We have found that the concentration of phosphate ion liberated from substrate by serum phosphatase action is nearly always directly proportional to time of incubation. This is illustrated in Fig. 2A. Forty-eight determinations were made on 16 sera whose phosphatase activity varied from 2.8 to 155.5 units. Times of incubation ranged from 2.5 minutes to 3 hours. In each experiment the phosphatase reading which had the phosphate concentration nearest to that of the standard was arbitrarily assigned a value of unity, and its time of incubation was termed standard time (T_s). The ratios of other times of incubation (T_e) to standard time were then found and plotted against the corresponding ratios for the amount of phosphate liberated by phosphatase ($P\text{-}ase_e/P\text{-}ase_s$). The reason for employing this time of incubation instead of 1 hour as the standard is that, with very high phosphatase values, incubation for 1 hour results in the liberation of phosphate in concentrations too high to be read in the colorimeter. In the figure, experimental values are represented by crosses and the theoretical ratios by the solid line. It is seen that only 3 of the points deviate from the theoretical line by more than the experimental error of the colorimetric method when the phosphate concentration of the filtrate is between one-fourth and 3 times that in the standard.

For tissue phosphatase the proportionality between time of incubation and concentration of phosphate liberated holds only when the final phosphate concentration is between one-half and three-halves that of the standard. This is illustrated in Fig. 2B, which comprises 19 determinations of acid and alkaline phosphatase on 6 tissue extracts. The phosphatase values ranged from 1.7 to 15.5 units per gm., and the times of incubation from 2.5 minutes to 2 hours.

This behavior of tissue phosphatase is similar to that reported (2) for serum. It is not apparent why, for the majority of sera, we fail to confirm Bodansky's findings, nor why, in our hands, serum phosphatase and tissue phosphatase behave differently. It is possible that under some conditions the products of hydrolysis tend to inhibit phosphatase activity, and that these conditions are realized regularly in tissue extracts but only rarely in serum. The mechanism of inhibition is unknown and deserves further study.

It is impossible to determine experimentally what the readings on extracts containing 0.10 units per gm. or 500.0 units per gm. would be after incubating

1 hour. In practice, therefore, we incubate both tissue extracts and sera for whatever times will give convenient final phosphate concentrations. We then calculate the number of milligrams of phosphorus liberated per gram of tissue during the incubation period employed and multiply the result by the quotient of 60 divided by the number of minutes of incubation. Thus our phosphatase unit, while reduced to the value at 60 minutes for convenience, is based primarily on the time required to liberate a given amount of phosphate, rather than on the amount of phosphate liberated in given time.

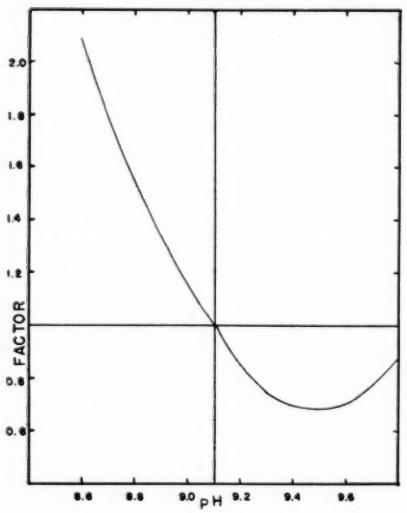


FIG. 1.—pH correction curve for serum alkaline phosphatase. Abscissas, pH at which determinations are made.
Ordinates, factors for correcting activities at experimental pH to activities at pH 9.10.

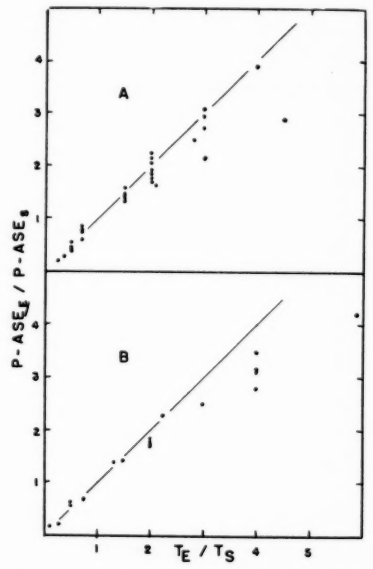
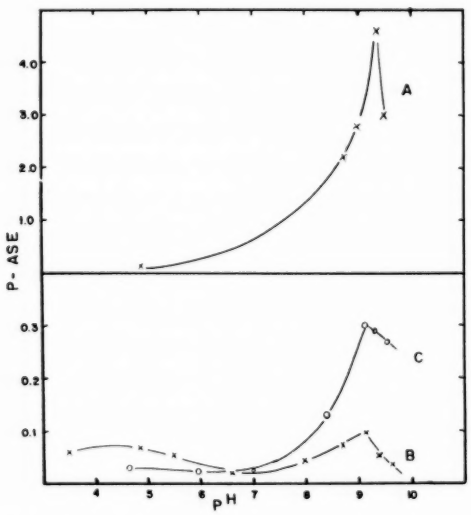
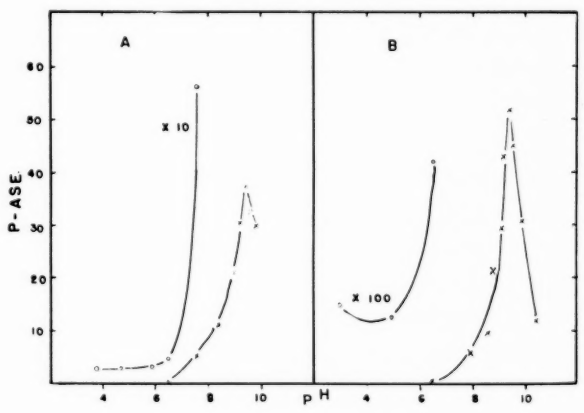


FIG. 2.—Relation of concentration of phosphate liberated to time of incubation. P-ase$_e$/P-ase$_s$ = ratio of concentration at experimental time to concentration at standard time. T_e/T_s = ratio of experimental time to standard time.





FIGS. 3 and 4.—Tissue phosphatase activities in units per gram plotted against pH at which determination is made.

FIG. 3.—Normal bone.
A. Cortex of normal growing radius.
B. Cortex of normal adult femur.
C. Cancellous portion of young adult tibia.

FIG. 4.—Pathological bone. Acid phosphatase plotted on scales 10 and 100 times that of alkaline phosphatase.
A. Curettings from os calcis showing bone rarefaction and fibrosis.
B. Osteogenic sarcoma, femur.

DILUTION

In general, as explained above, we employ a dilution of 9 volumes of substrate to 1 volume of serum or tissue extract. With very high phosphatase activities, such as are sometimes encountered in the serum of patients with Paget's disease, or in extracts of

prostatic or osteogenic sarcoma tissue, it is impossible to obtain satisfactory readings without further dilution. We have tested the effect of dilution on a few osteogenic sarcoma extracts, the time of incubation being varied inversely with the concentration, so that all final phosphate concentrations would be of the same order of magnitude. Results indicated that there is about a 20 per cent decrease in the phosphatase activity per gram of original tissue with a tenfold increase in dilution, but that a twofold change in dilution causes no significant change in activity. The effect of dilution on different preparations is not necessarily the same, since it is probable that phosphatase activity is influenced by coenzyme systems whose nature is not known at present, but whose activities may be affected by dilution in a different manner from that of phosphatase itself. It is recommended, therefore, that dilutions of phosphatase preparations be changed as little as is compatible with the use of accurately measurable times of incubation.

Effect of Standing

It has been shown by Bodansky (2) and confirmed by us (13) that the alkaline phosphatase activity of serum increases significantly on standing. Hence, we always determine serum phosphatase as soon as possible after the blood is drawn, and never preserve the specimens more than 18 hours. In contrast to serum phosphatase, the phosphatase activity of tissue extracts changes little on standing in the icebox for periods up to 2 months, provided no precipitate forms. This is illustrated in the following figures:

Tissue	Time of standing, days	Alkaline phosphatase, units per gm.
Osteogenic sarcoma	1	22.0
" "	15	18.8
" "	39	19.2
" "	59	22.0
" "	79	20.1
		Acid phosphatase, units per gm.
Adenocarcinoma	5	1.62
" "	13	1.67
" "	23	1.58
" "	40	1.45

Many extracts of soft-part tissues flocculate after a week or 10 days. The phosphatase adsorbs on the precipitate and, as this usually cannot be redispersed evenly by shaking, attempts at determining the activity of such extracts lead to erratic results. Highly turbid extracts which seem likely to flocculate should, therefore, be studied within 3 or 4 days of preparation. Examination of clear extracts, especially those of bone, may, on the other hand, be deferred for 1 or 2 weeks when desired.

RESULTS

Bone Phosphatase

When phosphatase activities are determined at different acidities and alkalinities and the activities are plotted against the pH values at which they were made, curves are obtained which are to some extent characteristic of the tissue of origin. Particularly is this true of bone phosphatase. This is illustrated in Fig. 3 by the phosphatase-pH curves for extracts of normal growing bone and of adult bone; and in Fig. 4 by similar curves for fibrotic hyperplastic bone and osteogenic sarcoma. It is seen that there is a sharp maximum in the alkaline range. The exact position of this maximum is hard to determine accurately, owing to the difficulty of securing exactly the desired pH, but it is nearly always close to pH 9.40. This is very near to the pH of maximum activity of the alkaline phosphatase of serum as shown in Fig. 1, and lends further weight to the evidence of numerous workers that the major portion of the alkaline phosphatase of serum is of osseous origin.

For the sake of uniformity, it was felt to be desirable to retain the same pH of reference (pH 9.10 electrometric or pH 8.6 colorimetric) for alkaline tissue phosphatase as that established by Bodansky (2) for serum. It is fortunate that this pH is not that of the maximum activity of bone phosphatase, since it would be too time-consuming to locate this point accurately as a routine procedure. It is comparatively easy, however, by determining phosphatase at approximately pH 9.00 and 9.20, to find the value at pH 9.10 by interpolation. It must be borne in mind, however, that the values for alkaline phosphatase reported in this paper are in general lower than the maximum obtainable.

While it is obvious that the bone extracts illustrated in Figs. 3A and 4 had an enormously greater phosphatase activity in alkaline solutions than in neutral or acid solutions, yet such extracts nearly always contain a small amount of acid phosphatase. In Fig. 4 the acid phosphatase values are plotted both on the same scale as the alkaline and on a scale 10 or 100 times as large. Inspection of the large-scale plot shows that the alkaline phosphatase is active down to pH 6.0, and that in still more acid solutions a true acid phosphatase makes its appearance. The presence of this acid phosphatase is more easily demonstrated in extracts of normal bone in which the alkaline phosphatase activity is low. This is illustrated in Fig. 3B and 3C by the phosphatase-pH curves for extracts of shaft of adult femur and epiphysis of young adult

fibula. The alkaline phosphatase activities are low, especially in the femur, in which the activity is only about 1/500 that of the osteogenic sarcoma shown in Fig. 4B, and the maxima are less sharp than in the more active extracts. The presence of an acid phosphatase with a broad maximum between pH 4.0 and 5.0 is easily seen. This acid phosphatase probably originates in nonosseous elements in bone or in interstitial fluid. The values for acid phosphatase reported in this paper were all determined at the pH of maximum activity.

Normal Bone

A sufficient number of specimens of healthy human bone have been examined to permit the establishment of normal values for alkaline phosphatase. These are summarized below. As no significant differences were found among the various long bones, values for femur, tibia, fibula, humerus, ulna, radius, and clavicle are grouped together.

Bone	Number of specimens	Alkaline phosphatase: Average, units per gm.	Alkaline phosphatase: Range, units per gm.
Adult long bone, cortex	12	0.04	0.01–0.15
" " " cancellous portion	5	0.14	0.05–0.30
Children's long bone, cortex	10	0.74	0.16–3.3
" " " cancellous portion	3	...	0.67–1.9
Adult rib cortex	6	0.49	0.23–0.88

Acid phosphatase activities were studied on 15 of the 36 specimens. Barely detectable amounts (0.01 to 0.03 units per gm.) were found in the cortex of long bone, whereas cancellous bone and rib contained 0.10 to 0.40 units per gm. This acid phosphatase probably originated in the marrow, small portions of which were unavoidably included in the bone selected for extraction.

The wide range of values encountered in children's bones undoubtedly reflects variations in the growth rate. In the cortex of adult long bone the alkaline phosphatase activity is exceedingly low, while in the adult rib it is 4 to 10 times as high. As the function of phosphatase in nongrowing bone is presumably to provide phosphate for the repair of ordinary wear and tear, we may infer that the continual bending strains to which ribs are subjected makes necessary a much more active repair mechanism than that of long bone. In adult long bone we feel that an alkaline phosphatase activity of 0.5 units per gm. or more indicates that the bone has recently been subjected to trauma or that some pathological process is at work.

A few determinations on flat bones and vertebrae indicate that their phosphatase activities are higher than those of other parts of the skeleton. Sufficient data are not yet at hand for the establishment of normal values for these bones.

The greatly increased alkaline phosphatase activity manifested in bone which is the site of an attempt at repair is illustrated in Fig. 4A. Five specimens of hyperplastic bone have been examined. In 3 the diagnosis was "fibrosing osteitis," in 1 "bone rarefaction and fibrosis," and in 1 "osteomyelitis." The alkaline phosphatase ranged from 1.8 to 28.5 units per gm., and the acid phosphatase from 0.10 to 0.28 units per gm.

Bone Tumors

Tissue phosphatase activities have been determined on 33 specimens of osteogenic sarcoma from 25 patients. None of these tumors had received preoperative irradiation. The alkaline phosphatase readings ranged from 0 to 115.0 units per gm. Acid phosphatase readings were made on 18 of the specimens and ranged from 0.02 to 0.80 units per gm. In the majority of cases the phosphatase-pH curves were definitely of the bone type shown in Fig. 4. This was true not only of most of the primary tumors but also of the 3 specimens of metastases to lung. There were, however, all gradations between this type of curve and one closely resembling that found for the giant cell tumors and endothelioma of bone to be discussed later. In several a wide difference in the phosphatase activities of different portions of the same tumor was found. Dr. F. W. Stewart of this Hospital has kindly reviewed the slides on many of these specimens and has been unable to find any correlation between histological type and phosphatase activity. We conclude, therefore, that the alkaline phosphatase activity of osteogenic sarcoma tissue reflects the functional state of the tumor at the time of examination regardless of the tissue elements which predominate. The possible implications of this observation have been discussed in more detail elsewhere (16).

Four specimens of osteochondroma were examined. The values for acid and alkaline phosphatase found were only slightly above those which would have been

expected in the normal bones from which these benign, slow-growing tumors arose.

Alkaline phosphatase determinations were made on 10 specimens of benign and malignant giant cell tumors. The alkaline phosphatase values ranged from 0.10 to 2.3 units per gm., but were above 0.50 units in only 4 specimens. All of these were atypical or frankly malignant. The onset of malignant tendencies is not necessarily associated with a rise in the alkaline phosphatase. Acid phosphatase determinations were made on only 4 of the specimens of giant cell tumor. In these the acid phosphatase values were found to range from 0.52 to 1.9 units per gm. While further work is necessary for definite conclusions, it seems probable that giant cell tumor tissue itself contains principally acid phosphatase. Where considerable amounts of alkaline phosphatase are found, this probably originates in the adjacent intact bone and is a manifestation of an attempt at repair such as takes place when the bones are invaded by metastases from tumors of soft-part origin.

We have had very little opportunity to examine active tissue from endothelioma of bone because this tumor is nearly always treated by roentgen irradiation before being removed surgically. We have, however, obtained 4 postmortem specimens of metastases and one untreated primary tumor. The acid phosphatase values of these were from 0.30 to 1.5 units per gm. The only specimen containing more than 0.15 units per gm. of alkaline phosphatase was a metastasis to rib with considerable new bone formation. The curve for this tissue, together with that for a soft-part metastasis from the same patient, appears in Fig. 5. The 2 curves are quite different. This is in contrast to the behavior of osteogenic sarcoma in which the metastases, even when not calcified, usually have phosphatase-pH curves indistinguishable from that of the primary. The situation in endothelioma of bone, which arises from endothelial rather than osseous elements, is probably analogous to that just discussed for giant cell tumor. Osteogenic sarcoma, on the other hand, arises from osseous tissue, and this origin is reflected in the capacity of most of the primary tumors and their metastases to manufacture alkaline phosphatase.

Muscle

In extracts of 6 specimens of normal skeletal muscle and 1 specimen of normal uterine muscle no alkaline phosphatase activity was found. Four of these specimens had barely detectable activities in the acid range (less than 0.05 units per gm.). Evidently the β-glycerophosphatases measured by the method employed here are not concerned in the mechanism for carbohydrate metabolism in muscle. Two specimens of uncalcified uterine fibroid behaved like normal muscle. The findings on 5 specimens of abnormal muscle and tumor arising from muscle are of interest and are summarized below.

Tissue	Acid phosphatase, units per gm.	Alkaline phosphatase, units per gm.
Atrophic fibrotic muscle	0.0	0.21
Edematous muscle	0.06	0.28
Spindle cell sarcoma probably of muscle type; calcification in tumor, not true ossification	0.0	1.0
Same tumor, soft necrotic area	0.15	0.95
Myositis ossificans showing dead bone in tendon or aponeurosis	0.10	8.7
Adjacent muscle, normal in the gross	0.03	0.03

The first 2 specimens had undergone mild pathological changes such as sometimes lead to calcification, and contained small but significant amounts of alkaline phosphatase. The third and fourth specimens were of a malignant tumor arising in, and probably from, muscle. One was calcified and the other not, but both contained abundant alkaline phosphatase. In the fifth specimen, which was from an area of myositis ossificans in an 8-year-old boy, the muscle had been entirely converted into bone. This abnormal bone had a higher alkaline phosphatase activity than we have observed in any normal bone, even in growing children. Only questionable alkaline phosphatase activity was found in the adjacent uninvolved muscle of the same patient. Wilkins, Regen, and Carpenter (12) have reported the presence of high alkaline glycerophosphatase activities, not only in calcified myositis ossificans tissue but also in muscle in the pre-ossification stage. These findings suggest that a derangement of the phosphorylation mechanism of muscle leading to the appearance of alkaline glycerophosphatase precedes calcification in ossifying muscle lesions, and may be the primary cause of these disturbances. Further studies on such tissues will be made as they become available.

Liver and Kidney

As stated in the introduction, it is known that liver and kidney contain both acid and alkaline phosphatase. Phosphatase-pH curves for specimens of these 2 tissues are given in Fig. 6. We have only a small series of observations on human liver and kidney, but have encountered considerable variation in both the relative and absolute amounts of acid and alkaline phosphatase, with consequent variations in the shapes of the curves. While the tissues were normal in the

gross, they were obtained from patients who had died of cancer, and the profound nutritional disturbances which preceded death may well have altered the metabolism of the liver and kidney. Hence, we can make no statement as to the phosphatase of these organs in normal man. We have, however, begun, in collaboration with Dr. K. Sugiura of this Hospital, an investigation of the livers of rats on a diet containing butter yellow. Preliminary observations indicate that the onset of malignant changes is associated with definite increase in phosphatase activity, especially in the alkaline range.

Prostate

While the presence of an enormous acid phosphatase activity in extracts of human prostate gland and of prostatic carcinoma is too well known to require detailed consideration here, it is of considerable theoretical and practical interest to determine the exact pH range over which this phosphatase is active. In Fig. 7A is presented the phosphatase-pH curve for an extract of a prostate showing benign hypertrophy and chronic interstitial prostatitis. In Fig. 7B is shown a similar curve for a fibromyomatous prostate. The left-hand portion of one curve and the right-hand portion of the other are also shown on a larger scale. There are small differences between the 2 curves, but in both there is a broad maximum between pH 4.0 and 5.5. At pH values lower or higher than this, the activity decreases rapidly. It is small below pH 2.0 and above pH 8.0 and disappears entirely above pH 8.5.

Testes

The alkaline phosphatase of 4 testes normal in the gross was found to range from 0 to 0.11 units per gm., and the acid phosphatase from 0.23 to 1.3 units per gm. As only one of the specimens was from a young man, the relation of phosphatase to functional activity is not known. In contrast to these findings on normal testis, the alkaline phosphatase reading on a primary embryonal adenocarcinoma of the testis was 1.3 units per gm., and the readings on 2 metastatic deposits from a similar tumor in another patient were 19.5 and 27.5 units per gm. The acid phosphatase of the 3 specimens ranged from 0.65 to 1.4 units per gm., or not much higher than in normal testes. The discovery in testicular tumors of an alkaline phosphatase activity higher than that which has been observed to date in any other tissue except regenerating bone and osteogenic sarcoma is entirely unexpected and will be studied further.

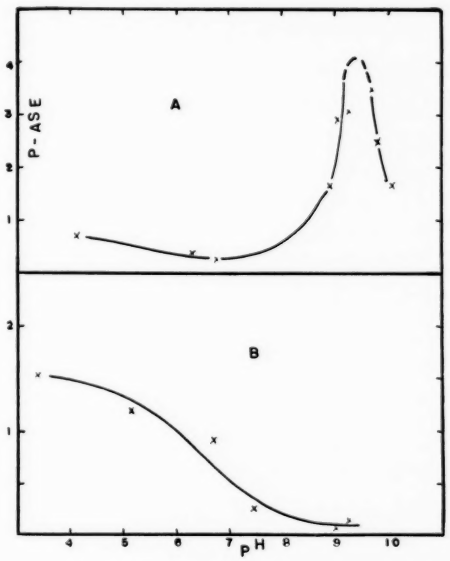
Carcinoma and Lymphomatoid Tissue

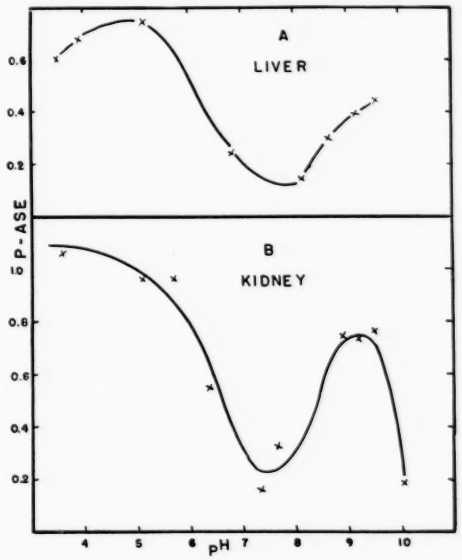
We have examined 7 specimens of carcinoma tissue and 6 of lymphomatoid tissue. In all, the phosphatase-pH curves were of the double type found for liver and kidney. The acid phosphatase figures ranged from 0.23 to 1.6 units per gm. The alkaline phosphatase activities averaged considerably lower and exceeded 0.40 units per gm. in only 2 cases. One of these was an adenoid cystic adenocarcinoma of salivary gland origin with 1.7 units per gm.; the other was lymphomatoid tissue from a patient with Hodgkin's disease and had an alkaline phosphatase activity of 0.68 units per gm.

The curves for 2 specimens of myeloid myeloma tissue, one invading muscle and one invading bone, appear in Fig. 8. The patient was a man of 50 years. The disease apparently originated in the marrow of the clavicle and extended through the cortex of the bone and into the soft parts. The soft mass, which was free of bone and muscle, contained 0.70 units per gm. of acid phosphatase and only 0.15 units per gm. of alkaline phosphatase. The mixed tissue consisted of a spongy framework of regenerating bone enclosing myeloma. The acid phosphatase activity of the extract of the mixture was about the same as that of the pure myeloma. It is evident that this came from the myeloma and not from the bone, since the activity of extracts of normal clavicle in the acid range is extremely low. This finding is analogous to the demonstration (8) of the presence of prostatic phosphatase activity in metastases to bone from carcinoma of the prostate, the only difference being in the very much smaller acid phosphatase activity of the myeloma tissue. The alkaline phosphatase activity of our bone specimen amounted to 3.6 units per gm. When it is remembered that normal adult clavicle does not contain more than 0.50 units per gm. of alkaline phosphatase, and usually much less, it is evident that the presence of a tumor tending to destroy bone resulted in a great increase in the activity of the repair mechanism of the osseous tissue. We have made similar observations on tissue from metastatic areas in bone from carcinoma of the breast. The curves in Fig. 8 are also similar to those in Fig. 5 for endothelioma of bone. It is, therefore, a frequent finding that bones which are the site of metastases from tumors of soft-part origin produce alkaline phosphatase in amounts far in excess of those produced in similar normal bones. This constitutes a direct proof of the theory which we postulated (15) from the indirect evidence obtained by the study of phosphatase in the serum of patients with metastatic bone disease.

Serum

The phosphatase-pH curves for the serum of a normal young woman and of a normal young man appear in Fig. 9A and 9B, respectively. We have obtained similar curves from numerous normal individuals and have not observed significant differences between males and females. Besides the well-known maximum of activity at about pH 9.5 there was in all curves a broad maximum between pH 4.0 and 5.0 and a minimum at about pH 6.5. Absolute values in the acid range were low. For a series of 12 females and 13 males in whom the alkaline phosphatase did not exceed 5.0 units per 100 cc., the average acid phosphatase determined between pH 4.0 and 5.0 was 0.28 units per 100 cc., with a range of 0.04 to 0.64 units per 100 cc. The group included 9 normal persons and 16 patients with miscellaneous diseases not involving the prostate gland. There was no significant

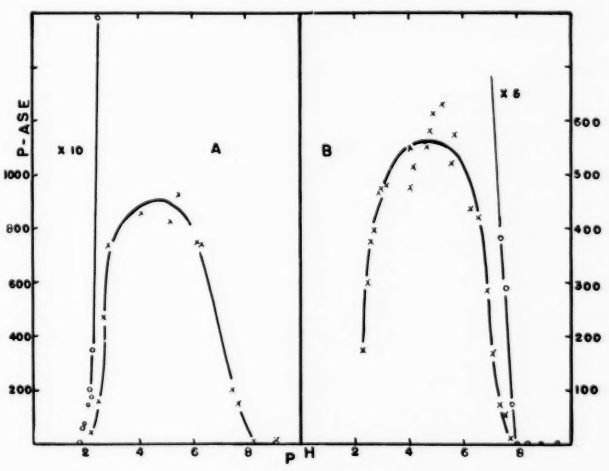




Figs. 5 to 8 inclusive.—Tissue phosphatase activities in units per gram plotted against pH at which determination is made.

Fig. 5.—A. Metastasis to bone from endothelioma of bone (Ewing's tumor).
B. Metastasis to soft parts from same tumor.

Fig. 6.—A. Normal liver.
B. Normal kidney.



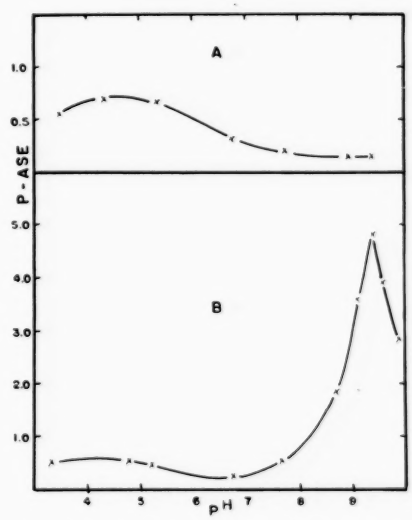


Fig. 7.—Prostate.
A. Benign hypertrophy and chronic interstitial prostatitis. Left hand portion of curve plotted on scale 10 times that of remainder.
B. Fibromyomatous prostate. Alkaline phosphatase plotted on scale 5 times that of acid phosphatase.

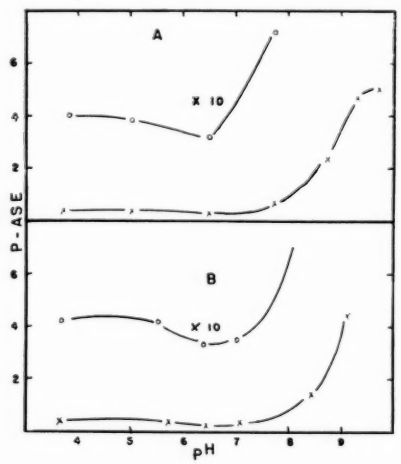
Fig. 8.—A. Uncalcified myeloid myeloma.
B. Clavicle invaded by myeloid myeloma.

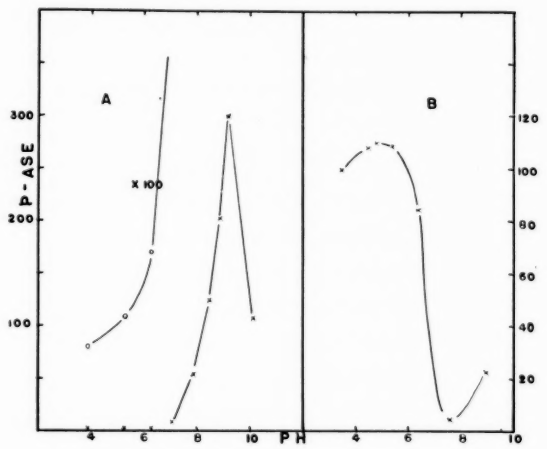
difference in the average and range of readings for males and females. It is evident that human serum contains small amounts of acid glycerophosphatase which are not derived from the prostate gland. This is in harmony with the findings of the Gutmans (5) for phenylphosphatase.

It will be remembered from Fig. 7 that the activity of extracts of prostatic tissue at pH 6.5 is only 20 to 30 per cent less than that at pH 4.5, while the activity of bone phosphatase at pH 6.5 is commonly less than 10 per cent of that at pH 9.1. The activity of the mixture of phosphatases in normal human serum is at a minimum at pH 6.5, although this activity is only about 30 per cent less than that at the acid maximum. When the minimum of activity of normal serum at pH 6.5 was first discovered, it was thought that this was the point at which the presence of prostatic phosphatase could be demonstrated with the least confusion from alkaline phosphatase or nonprostatic acid phosphatase. We employed this pH of reference for about 200 determinations of acid serum phosphatase and found the results useful in diagnosing metastatic carcinoma of the prostate. The method was not entirely satisfactory because, in patients with very high alkaline serum phosphatase readings, the readings at pH 6.5 were not clear-cut owing to residual alkaline phosphatase activity at this pH. In the attempt to eliminate this difficulty we have now adopted pH 4.5 as our reference point for acid serum phosphatase.

Even at pH 4.5 we have not been completely successful in avoiding the effects of alkaline phosphatase. In patients without prostatic cancer, in whom the alkaline phosphatase was normal, we have not encountered serum phosphatase activities at pH 4.0 to 5.0 as great as 0.7 units per 100 cc. But in 5 of 25 patients in whom the alkaline phosphatase was elevated the acid phosphatase was from 0.7 to 0.9 units per 100 cc. The phosphatase-pH curve for the serum of one of the cases is given in Fig. 10A. The patient was a man of 40 with advanced osteitis deformans. Prostatic carcinoma could be excluded not only by the physical and roentgenographic findings but also by the long history. The acid phosphatase readings are plotted both on the same scale as the alkaline and on a scale 10 times as large. There is evidence that the alkaline phosphatase was active at a pH at least as low as 5.0, and probably somewhat lower. It thus appears impossible to exclude residual alkaline phosphatase activity entirely except at a pH below that of maximum activity for acid phosphatase. In practice we find it most satisfactory to determine the acid serum phosphatase at pH 4.5 and make allowance for possible effects of alkaline phosphatase in interpreting the results.

In order to demonstrate that the behavior of prostatic phosphatase with respect to the pH of the substrate is the same in serum as it is in extracts of prostatic tissue, we have prepared the curve for serum phosphatase in Fig. 10B. The patient was a man of 52 with a moderate degree of bone involvement and very extensive soft-part metastases from carcinoma of the prostate. It is seen that the left-hand position of the curve resembles very closely those for prostatic extracts in Fig. 7. The right hand portions are of





FIGS. 9 and 10.—Serum phosphatase in units per 100 cc. plotted against pH at which determination is made.

FIG. 9.—A. Serum phosphatase, normal female.
B. Serum phosphatase, normal male.
Acid phosphatase plotted on scale 10 times that of alkaline.

FIG. 10.—A. Serum phosphatase, osteitis deformans. Acid phosphatase plotted on scale 100 times that of alkaline phosphatase.
B. Serum phosphatase, metastases to bone and soft parts from carcinoma of the prostate.

course different, as the serum contains considerable alkaline phosphatase originating in the regenerating areas of the involved bones.

The clinical significance of our determinations of acid serum phosphatase will not be discussed in detail here. In summary, we believe that the presence of a serum phosphatase activity at pH 4.5 of 0.7 to 1.0 units per 100 cc., when the alkaline phosphatase is normal, warrants the suspicion that the patient has metastasizing carcinoma of the prostate; and we feel that this diagnosis is very probable when the acid phosphatase exceeds 1.0 units per 100 cc. If the alkaline serum phosphatase is elevated, then a phosphatase activity at pH 4.5 of 1.5 units per 100 cc. or more appears to be pathognomonic of metastasizing carcinoma of the prostate. In doubtful cases the acid phosphatase may be determined at both pH 4.5 and pH 6.5. If the reading at pH 4.5 is higher than that at pH 6.5, then there is probably a true elevation of acid phosphatase rather than a high residual activity of alkaline phosphatase in the acid range. If the acid serum phosphatase is elevated and the alkaline phosphatase is normal, the patient probably has carcinoma of the prostate metastatic to soft parts but not to bone. If both acid and alkaline phosphatases are elevated, there are metastases to bone from carcinoma of the prostate. If both phosphatases are elevated and the acid is higher than the alkaline, it is likely that there are prostatic metastases to both bone and soft parts. We have not seen significant elevations in the acid serum phosphatase of patients with carcinoma of the prostate without metastases. On the other hand, the presence of a normal acid serum phosphatase gives no assurance that metastases from carcinoma of the prostate are not present.

SUMMARY

A method is described for measuring the action of serum and of crude tissue extracts on sodium-β-glycerophosphate over the pH range from 3.0 to 10.0.

The alkaline glycerophosphatase activity of the cortex of normal adult human long bone has been found to range from 0.04 to 0.15 units per gm. Corresponding values for children's long bones are 0.16 to 3.3 units per gm. Acid phosphatase activities in cortical bone were barely detectible.

Regenerating bone has been found to contain up to 50 times as much alkaline glycerophosphatase as normal bone.

A range of alkaline glycerophosphatase activity from 0 to 115.0 units per gm. has been found in extracts of osteogenic sarcoma tissue. No correlation has been found between phosphatase activity and histological type. Soft-part metastases from osteogenic sarcoma have alkaline glycerophosphatase activities of the same order of magnitude as the primary tumor.

The glycerophosphatases of extracts of giant cell tumors and of endothelioma of bone are active mainly in acid solution. Significant alkaline glycerophosphatase activities are found only when portions of regenerating bone are included in the specimen.

Areas of bone invaded by metastases from tumors of soft part origin contain more alkaline glycerophosphatase than does normal bone.

Acid and alkaline glycerophosphatase activities of the order of 0.5 to 1.5 units per gm. have been found in extracts of normal liver and kidney, and in most specimens of carcinoma and lymphomatoid tissue.

No alkaline glycerophosphatase and very small amounts of acid glycerophosphatase have been found in extracts of normal muscle. Significant amounts of alkaline glycerophosphatase have been found in a few extracts of pathological muscle.

Some evidence has been obtained that the alkaline glycerophosphatase of embryonal adenocarcinoma of the testis is much higher than that of normal testis.

Prostatic glycerophosphatase has been found to be active from pH 2.0 to 8.0. Slight activity was occasionally detected at a pH as high as 8.3, but none in solutions more alkaline than this.

The significance of acid phosphatase readings on the serum of patients with carcinoma of the prostate is discussed.

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The Utilization of Vitamin C by Cancer Patients*

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Vitamin C occurs in high concentration in all actively growing parts of the higher plants (21), in the key endocrine organs (5, 6, 21), and in young animal and human tissues (6, 37). A direct relationship exists between the functional activity of the corpus luteum and its content of vitamin C (7). An adequate amount of this substance seems essential for normal wound healing (22). Vitamin C thus appears to be associated with the growth and functional activity of plant and animal tissues.

It seemed possible that the increased metabolic activity of carcinomatous tissue might be associated with an increased local utilization of vitamin C which, if of sufficient magnitude, would cause a detectable decrease in the urinary excretion of this substance by cancer patients.

The present study was undertaken in an endeavor to investigate the validity of this hypothesis. It consists of a quantitative estimation of the utilization of vitamin C by cancer patients, with suitable controls.

There are no direct data in the literature establishing the degree of vitamin C utilization in cancer. Human and animal tumor tissues appear in general to have a higher concentration of the vitamin than normal tissues, although exceptions occur (3, 8, 34, 36). The concentration in rat hepatoma was found to be several times greater than in normal rat liver tissue (14). It is of interest that the concentration in tetraploid strains of the tomato was observed to be approximately twice that of the diploid strains from which they were derived (27).

Reports differ concerning the effect of tumor implantation on the vitamin C concentration of animal tissues, some observers finding the concentration unchanged (34, 36), others finding it decreased (30, 33). There is also disagreement on the effect of the vitamin on tumor growth, some finding no effect (24, 36), others a stimulatory action (17, 32, 35).

Studies in man have revealed a subnormal excretion of ascorbic acid in response to test doses in the majority of cases of advanced malignancy (2, 15, 28), and an increased concentration of the substance in whole blood in leukemia (29).

METHOD OF STUDY

Thirteen patients were studied, 5 with metastatic cancer, 1 with localized cancer, and 7 controls, for periods of from 9 to 16 days each. The pertinent clinical data are shown in Table I. All patients were ambulant adult males. All had normal blood nonprotein nitrogen levels and negative routine urinalyses, except case 1, who had a constant 2-plus albuminuria; his urea clearance and dye excretion tests were normal. None had leucocytosis or fever, except case 9, who had had a cholecysto-gastrostomy for biliary obstruction 10 months previously and who had sudden transitory febrile spikes to 104° F. on the 2nd and 5th postsaturation days. There was no reasonable doubt as to the diagnosis of malignancy in the cancer group, which was established by exploratory laparotomy in cases 8, 12, and 13, by biopsy in cases 9 and 11, and by postmortem examination in case 10.

A fruit-free, vegetable-free diet was given.

Ammonium chloride, 4 gm., was given daily to ensure an acid urine and enhance the recovery of vitamin C (18). Alkali administration was forbidden.

Vitamin C,[1] 500 mgm., was injected daily subcutaneously. Parenteral administration was selected in preference to oral because of partial loss of the vitamin in the digestive tract by destruction, neutralization, or malabsorption (1, 20); the subcutaneous route permitted slow absorption, minimizing the plasma elevation and consequent overflow through the kidneys which occurs when large doses are given intravenously (19, 25), and because of the size of the dose (5 cc.) was more convenient than the intramuscular route.

Immediately prior to each injection, blood was withdrawn and the plasma ascorbic acid content determined according to the macromethod of Farmer and Abt (12, 13). At the same time, 24-hour urines were collected and their total vitamin C content was determined according to the method of Harris and Ray (16). The accuracy of these methods, which depend upon the reduction of indophenol, is considered comparable to that of blood sugar tests (5): A maximum error of perhaps 5 per cent may occur.

Each daily urine specimen was collected in a large dark brown bottle containing 100 cc. glacial acetic acid, which was stored in an icebox and into which the patient voided directly. Preliminary experiments with added crystalline ascorbic acid showed that under these conditions approximately 3 per cent was lost during 24 hours. The values obtained for daily ascorbic acid excretion were therefore corrected for this loss.

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[1] The vitamin C used in this investigation was vitamin C forte, injectable, Roche, kindly supplied by Hoffmann-La Roche, Inc., Nutley, N. J.

TABLE I: CLINICAL DATA

Case	Diagnosis	Metastases	Age	Weight, kilograms	Height, inches	Basal metabolic rate
		CONTROL CASES				
1	Nephrosis	.....	40	59	68	−34
2	Tuberculous chest sinus	.....	59	80	66	−12
3	Benign stricture of esophagus	.....	48	54	66	−11
4	Dermatitis venenata	.....	61	54	64	+24
5	Malnutrition	.....	55	55	67	+ 1
6	Arteriosclerotic heart disease	.....	62	84	72	+ 9
7	Seborrheic dermatitis	.....	55	70	65	−12
		Average	54	65	67	− 5
		CANCER CASES				
8	Carcinoma of pancreas	None	70	52	70	−35
9	Carcinoma of papilla of Vater *	Regional lymphatic	45	77	71	+ 3
10	Carcinoma of sigmoid colon *	Hepatic	65	68	72	+27
11	Carcinoma of esophagus *	Distant lymphatic	76	46	63	− 5
12	Carcinoma of stomach *	Regional lymphatic	64	53	67	− 8
13	Carcinoma of stomach *	Hepatic	66	54	65	+ 2
		Average *	63	60	68	+ 4

* Cases with metastases.

TABLE II: URINARY AND PLASMA RESPONSES TO 500 MGM. VITAMIN C DAILY SUBCUTANEOUSLY

Days		Control cases								Cancer cases						
		1	2	3	4	5	6	7	Av.	8	9	10	11	12	13	Av.*
		URINARY ASCORBIC ACID, MGM.														
Before saturation	7	...	...	10	...	...	...	...	...	...	...	...	...	...	...	...
	6	...	...	30	...	...	...	...	...	...	...	...	...	...	...	...
	5	...	...	15	...	...	...	140	...	10	...	30	0	...	...	...
	4	...	...	15	...	...	...	325	...	35	...	40	10	10	...	...
	3	110	35	140	...	...	55		85	...	...	205	15	25	...	82
	2	265	175	260	...	410	230	395	289	...	345	170	210	75	...	200
	1	395	255	365	330	430	330	445	364	...	390	445	325	185	310	331
Saturation		630	500	415	455	445	425	450	474	400	505	510	410	380	425	446
After saturation	1	370	440	400	400	440	360	390	400	370	450	415	385	345	330	385
	2	515	390	375	480	450	420	380	430	440	265	435	345	300	410	351
	3	465	405	465	410	...	510	395	442	370	...	355	410	415	375	389
	4	550	395	450	455	535	450	415	464	450	395	410	335	465	250	371
	5	490	355	455	400	445	395	335	411	280	440	495	415	435	365	430
	6	530	665	470	440	285	400	380	454	470	...	230	390	370	305	324
	7	435	460	...	445	350	...	390	416	470	470	465	420	285	...	410
	8	360	570	...	...	450	...	560	485	510	430	315	415	220	...	345
	9	405	390	...	...	415	395	465	414	525	345	390	355	420	...	378
		PLASMA ASCORBIC ACID, MGM. %														
Before saturation	8	...	...	0.28	...	...	...	...	...	...	...	...	...	...	...	...
	7	...	...	0.34	...	...	...	...	...	...	...	...	...	...	...	...
	6	...	...	0.34	...	...	...	0.84	...	0.14	...	0.24	0.10	...	...	...
	5	...	...	0.54	...	...	...	1.28	...	0.26	...	0.40	0.18	0.14	...	...
	4	0.44	0.34	0.66	...	1.22	0.62	...	...	0.48	...	0.46	.	0.32	...	...
	3	0.66	0.28	...	...	1.28	1.24	1.56	1.00	0.88	1.42	0.56	0.84	0.56	1.44	0.96
	2	0.92	1.00	0.96	1.14	1.34	1.38	1.64	1.20	1.08	..	0.58	1.16	0.86	1.70	1.08
	1	0.90	...	1.00	1.16	1.28	1.34	1.64	1.22	1.12	1.38	0.80	1.42		1.94	1.39
Saturation		1.10	1.00	1.08	1.52	1.22	...	1.56	1.25	1.16	1.02	0.84	1.38	1.56	1.90	1.34
After saturation	1	0.94	0.98	1.06	1.36	...	...	1.54	1.18	0.94	1.16	0.84	1.38	1.56	1.96	1.38
	2	0.94	1.30	1.14	1.14	1.36	1.42	1.38	1.24	1.08	0.98	0.80	1.42	1.40	1.88	1.30
	3	1.02	1.02	1.00	1.18	...	1.44	1.44	1.18	1.14	1.06	0.88	...	1.40	1.76	1.28
	4	1.00	1.04	1.04	1.26	1.28	1.32	1.42	1.19	0.98	1.12	0.72	1.26	1.34	1.66	1.22
	5	0.94	1.02	1.06	1.20	1.34	...	1.46	1.17	1.02	1.04	0.68	1.26	1.22	1.48	1.14
	6	0.92	0.78	...	1.22	...	1.44	1.90	1.25	0.94	1.08	0.64	1.24	...	1.78	1.19
	7	1.02	...	...	...	1.08	...	1.64	1.25	1.06	1.06	0.64	...	1.42	1.68	1.20
	8	0.88	1.02	...	...	1.12	1.28	1.46	1.15	1.08	1.26	0.64	1.28	1.32	...	1.13
	9	0.94	0.78	...	...	1.06	1.44	1.52	1.15	0.98	1.16	0.68	1.34	1.26	...	1.11

* Cases with metastases.

RESULTS

The urinary and plasma responses to the injected ascorbic acid are recorded quantitatively in Table II, and graphically for the control and metastatic cancer groups in Fig. 1.

In each case the 24 hour urine values for vitamin C sooner or later increased until a peak was reached, following which a somewhat smaller excretion occurred which tended to be uniform. The plasma values assumed a similar curve, the initial peak occurring a day or two earlier than that for urine.

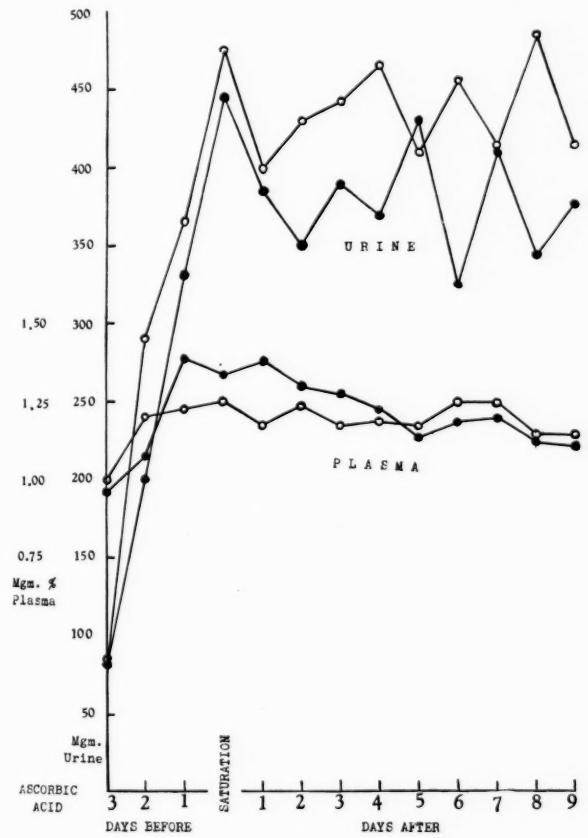

FIG. 1.—Composite urinary and plasma responses of metastatic cancer patients (●—●) and controls (o—o) to 500 mgm. of vitamin daily subcutaneously.

These findings are interpreted as indicating an initial tissue retention of the injected vitamin C, varying in degree with the size of the tissue reserves, until complete saturation had been achieved, shown by the initial excretion peak.

Following complete tissue saturation, the difference between vitamin C supply and excretion is considered to represent the vitamin C utilization in the body. Inasmuch as there is no evidence of endogenous supply in the human subject (21), the daily supply in this study comprised the 500 mgm. given parenterally plus an estimated 10 mgm. in the diet (5). The daily excretion comprised the amount eliminated *via* the urinary tract plus an estimated 10 mgm. eliminated *via* other channels (9, 10). The daily utilization of vitamin C was therefore calculated as 500 mgm. less the 24 hour urinary excretion; this latter value, because of daily fluctuations, was computed as an average of from 6 to 9 values in each case.

The daily utilization of vitamin C (Table III and Fig. 2) in the control group varied from 42 to 88 mgm., averaging 67 mgm. These values are not comparable with any reported in the literature, because of the different technic employed in their determination. In other studies, in which vitamin C has been given by mouth, the normal daily human requirement to maintain tissue saturation has been found to vary from 70 to 135 mgm. (4, 31).

The daily utilization of vitamin C in the patient with localized cancer was 68 mgm., a value falling well within the range of the control group.

The daily utilization of vitamin C in the metastatic cancer group varied from 101 to 161 mgm., averaging 125 mgm. This group, therefore, was found to utilize approximately twice as much vitamin C as the control group.

COMMENT

Before accepting unreservedly the finding of an increased utilization of vitamin C by patients with metastatic cancer, a critical appraisal of the data upon which it is based must be made. The wide fluctuations in postsaturation excretion values caused each value to assume undue importance in the determination of vitamin C utilization. These fluctuations may be attributed to the degree of co-operation of the patient, to errors inherent in the technic, and to normal daily physiological variants. Both groups of patients seemed equally co-operative; technical errors should have cancelled out. Other workers have also observed a considerable variation in urinary excretion for the same person even under carefully controlled conditions of ascorbic acid intake (31).

It is evident, furthermore, that about the same vitamin C supply was required for saturation by both groups: an average of 4.4 days or 2,200 mgm. for each patient. It might be anticipated that an increased usage of the vitamin in those with metastatic cancer would have lowered the tissue reserves and raised the saturation requirement—indeed, that manifest scurvy should appear eventually in carcinomatous patients. The primary importance of dietary intake, however, is emphasized with case 3, a patient whose low tissue reserves were presumably the result of a low intake occasioned by esophageal stricture. In this connection, it is significant that the plasma ascorbic acid of a normal man on a diet completely devoid of vitamin C fell from normal to zero after 41 days, following which 4 months elapsed before the appearance of

petechiae (11). Because of the possibility of gross inaccuracies, no record was made of the dietary histories of these patients.

The plasma values obtained at saturation and thereafter showed considerable individual variation, a finding which has been reported (1) and which can best be explained as indicative of variations in renal threshold. There was no correlation between individual plasma maintenance levels and the corresponding average urinary excretion of the vitamin. No relationship was evident between vitamin C utilization and age, body weight, height, or basal metabolic rate in these cases.

Considerable evidence has accumulated in support of the concept that ascorbic acid may function in animal tissues as a respiratory enzyme. It has recently been shown that this vitamin is essential for completing the oxidation of certain aromatic amino acids (23). In studies on the oxidation of rat liver phospholipid, it was observed that ascorbic acid possessed a marked catalytic action (26). Moreover, it was found that this action was inhibited by a number of carcinogenic agents, the degree of inhibition tending to vary directly with the carcinogenicity of the agent; all the carcinogens studied exerted this inhibitory effect.

A possible explanation for the finding of an increased utilization of vitamin C by patients with metastatic cancer is therefore apparent. If the presence of an active carcinogen in cancer patients is postulated, an interference with cellular respiration by this agent might cause a compensatory accumulation of ascorbic acid in the affected tissues and thus diminish the excretion of vitamin C.

SUMMARY

The daily utilization of vitamin C was investigated in 13 patients (7 controls, one patient with localized cancer, and 5 with metastatic cancer) and found to average 67 mgm., 68 mgm., and 125 mgm., respectively. The natural occurrence of vitamin C in high concentration in actively growing and metabolizing tissues, and the probable function of the vitamin as a respiratory enzyme, suggest that this finding may result from an accelerated usage of vitamin C by carcinomatous tissue.

TABLE III: DAILY UTILIZATION OF VITAMIN C

	Control cases							Cancer cases					
Case number	1	2	3	4	5	6	7	8	9	10	11	12	13
	ASCORBIC ACID, MGM.												
Postsaturation excretion	458	452	436	433	421	419	412	432	399	390	386	362	339
Utilization	42	48	64	67	79	81	88	68	101	110	114	138	161
Same, for group (case 8 omitted)				67							125		

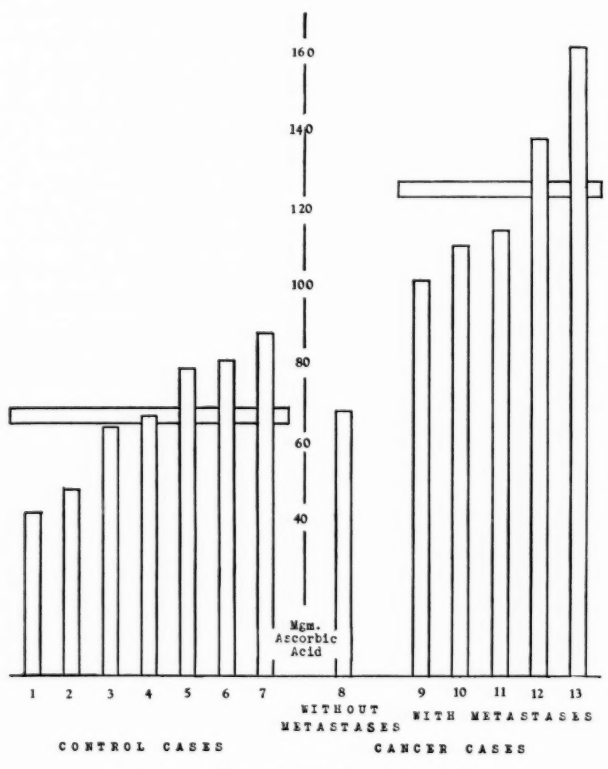

FIG. 2.—Daily utilization of vitamin C.

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Studies of the Transmissible Agent of Chicken Sarcoma I

Isolation of Virus from Basic Protein-Virus Complex*

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Previous experiments have shown that the tumor-producing agent or virus of chicken sarcoma I (Rous) can be precipitated with basic proteins, namely papain and histone (5) or protamine (6), and that the basic protein-virus complex was at least as active as the original extract of the sarcoma (5). However, it was found that the papain-virus complex was contaminated with other components, all of which could not be removed in spite of several redissolutions and reprecipitations of the complex.

Since the papain and virus form a complex probably through a salt linkage, a method of dissociation was sought in order to separate the two components and effect a purification of the virus. Solution of this water-insoluble complex was brought about by the addition of salt, indicating that dissociation of the complex had occurred. Separation of the virus from the basic protein was then possible by taking advantage of the high molecular weight of the virus. On high speed centrifugation of the dissociated complex, the active fraction was deposited free of papain and other components, as determined by electrophoresis.

EXPERIMENTAL

The methods of extraction, precipitation of the virus with papain, and purification of the formed complex described in the previous publication (5) were followed in most details. One kg. of fresh tumor was ground in a mortar with sand and extracted by stirring with a liter of 0.02 M phosphate buffer, pH 7.2–7.4 at 37° C., for 10 minutes. This was done in the presence of 100 mgm. of hyaluronidase isolated from sheep testis by the method of Chain and Duthie (1). The mixture was then cooled rapidly to about 10° C., further extracted by stirring for 1 hour, and centrifuged at 5,000 r.p.m. in the cold for about 30 minutes. The supernatant fluid was placed in the refrigerator overnight, since the experiment requires 2 days and the virus is stable in this solution.

The solution was then filtered through a Mandler filter or a layer of Hyflo super-cel. To each liter of filtrate (A) about 500 cc. of papain solution, containing 3 mg. N per cc., were added as previously described. The precipitate, papain-virus complex, was then purified in the following manner. It was centrifuged off in the cold, washed several times with cold water, and finally dissolved in about 150 cc. of 3 per cent NaCl solution. This solution was centrifuged in the cold and a small amount of undissolved material discarded. Cold water was added to the NaCl solution until a 10-fold dilution occurred and the precipitate which formed was centrifuged off, dissolved in 100 cc. of 3 per cent NaCl, and reprecipitated by dilution with cold water. Finally the precipitate was dissolved in about 75 cc. of 3 per cent NaCl solution. A portion of the purified papain-virus complex solution (B) was inoculated into chickens for the determination of activity.

The purified complex, which is soluble in saline because of dissociation, was then subjected to high speed centrifugation (about 20,000 r.p.m.) for 90 minutes. The supernatant fluid (C) of this centrifugation was similarly tested for tumor-producing activity. The formed pellets were dissolved in 30 cc. of 3 per cent NaCl and, after a small amount of insoluble material had been discarded, were recentrifuged at high speed for 90 minutes. The deposited material was then extracted with about 20 cc. of cold water or 0.02 M phosphate buffer, pH 7.4, and any insoluble material discarded after washing. The bulk of the material was soluble in water, showing that high speed centrifugation separated the virus material from the added papain. The extract and washings were combined (about 25 cc.) and centrifuged at high speed for 80 minutes. The formed pellets were dissolved in 20 cc. of water or buffer and the solution was centrifuged again at high speed for 70 minutes. The pellets (D) (150 to 250 mgm.) were finally dissolved in water and any insoluble material was washed and discarded.

* This investigation was aided by a grant from The International Cancer Research Foundation.

The activity of this final solution of the pellets (D) was determined and compared to the activities of the original filtrate (A), the thrice precipitated papain-virus complex (B), and the supernatant fluid (C) from the first high speed centrifugation. The activities were determined by inoculation of 10-fold dilutions of the test materials as described and the smallest amount of material (expressed as nitrogen content) which would give rise to tumors within 4 weeks was noted.

When enough of the final material (D) was available, its homogeneity was determined in an electrophoresis apparatus and a portion analyzed. For chemical analysis, the solution was frozen, the water distilled off in high vacuo, and the powdery material finally dried at 70° C. in vacuo.

Nitrogen was determined by micro-Kjeldahl method, phosphorus by the gravimetric method of Pregl, and purine nitrogen by the method of Graff and Maculla (3). Total fat was estimated by extracting the dried material with ether.

Comparison of activities.—From Table I one can see that the pellet deposited by high speed centrifugation of the papain-virus complex was in most cases as active as, or more active than, the original filtrate and the complex from which it was obtained. The supernatant fluid from the first high speed centrifugation was totally devoid of activity even in concentrations of 1×10^{-1} mgm. nitrogen.

Analysis.—The various preparations were found to be similar in composition, as shown in Table II. If one assumes that the purine determined is part of a tetranucleotide, the nucleic acid content can be calculated as 1.5 per cent of the total material (D) and 2.6 per cent of the nonlipid fraction. The nucleic acid phosphorus will then account for 10.6 per cent of the total phosphorus and 16.9 per cent of the total phosphorus in the nonlipid fraction.

Electrophoresis experiments.—The solution of the pellets (D), obtained by high speed centrifugation of the complex, was dialyzed to equilibrium against phosphate buffer 0.02 M, pH 7.4. On electrophoresis, the material was found to exhibit only one component (Figs. 1 and 2) and even after 2 hours the single peak did not show any resolution. The mobility of this peak, or component, in the presence of 0.15 M NaCl was 4.9×10^{-5} $\frac{\text{cm./sec.}}{\text{volts/cm.}}$, and 11.0×10^{-5} $\frac{\text{cm./sec.}}{\text{volts/cm.}}$ in the absence of sodium chloride. There was no evidence of papain in the final preparation.

TABLE I: COMPARISON OF MINIMUM ACTIVE DOSE OF VARIOUS FRACTIONS

Experiment No.	Filtrate (A), mgm. N	Papain-virus complex (B), mgm. N	Supernatant of pellet (C), mgm. N	Pellet (D), mgm. N
B15	3.7×10^{-3}	2.0×10^{-3}	2.0×10^{-2} (inactive)	5.0×10^{-5}
B20	2.8×10^{-5}	1.8×10^{-5}	1.7×10^{-1} (inactive)	7.0×10^{-7}
B22	3.2×10^{-4}	1.3×10^{-3}	1.2×10^{-1} (inactive)	5.0×10^{-4}
B23	3.5×10^{-5}	2.9×10^{-4}	2.9×10^{-1} (inactive)	1.6×10^{-6}
B24	3.0×10^{-5}	2.2×10^{-4}	2.0×10^{-1} (inactive)	6.0×10^{-6}
B25	3.0×10^{-6}	1.8×10^{-5}	———	3.0×10^{-5}
C1	3.3×10^{-4}	2.4×10^{-3}	———	3.7×10^{-4}
C2	3.4×10^{-4}	2.8×10^{-4}	———	1.3×10^{-5}
C3	4.3×10^{-6}	2.5×10^{-6}	———	1.4×10^{-6}
C6	3.8×10^{-5}	4.2×10^{-3}	———	4.2×10^{-5}
C7	3.9×10^{-5}	2.8×10^{-5}	———	6.0×10^{-6}
C8	3.8×10^{-5}	4.1×10^{-3}	———	1.3×10^{-3}

TABLE II: ANALYSES OF "VIRUS" PREPARATIONS

Experiment No.	Nitrogen, per cent	Phosphorus, per cent	Fat, per cent	Purine N / Total N, per cent	Nonfat residue: Nitrogen, per cent	Nonfat residue: Phosphorus, per cent	Fat fraction / Phosphorus, per cent
C1	7.2	1.58	42.0	2.1	11.2	...	...
C2	7.4	...	...	..	...	...	...
C7	7.2	1.56	43.5	2.5	11.7	1.69	1.26
C8	7.1	...	...	..	...	...	...

DISCUSSION

It is a difficult task to isolate a pure and homogeneous protein or, for that matter, to recognize it as such. This problem of homogeneity becomes even more difficult when the protein is only a part, or seems to be a part, of a molecule which exhibits a specific biological activity. When other components such as lipids, carbohydrates, or nucleic acids accompany proteins it is not always possible to decide whether the material is a true compound in which the various components are chemically linked, or only a mixture. In recent years, it has been recognized

that many biologically active materials are conjugated proteins.

Although the material isolated in the procedure described above was electrophoretically homogeneous at pH 7.4, one cannot infer that it is chemically pure and homogeneous and that the combination of the various components found is necessary for biological activity. The material should be subjected to many more rigorous tests before any conclusion is drawn. However, the material isolated by this technic was qualitatively similar to that prepared by Claude (2) and by Pollard (4), using only differential centrifugation. The percentages of fat and phosphorus of the material isolated in our experiments were similar to that reported by these authors, but the nitrogen content of the whole material and the nonlipid fraction was lower in our preparations. The nucleic acid content, calculated from the purine N determination, in this report, was much lower than the amount Claude estimated in his fractions (2).

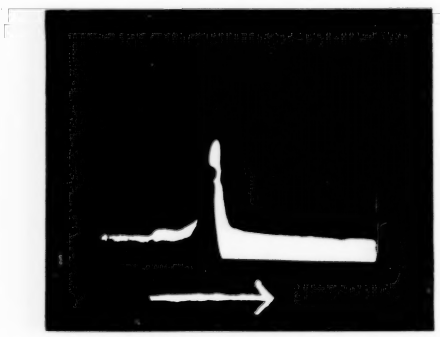

FIG. 1

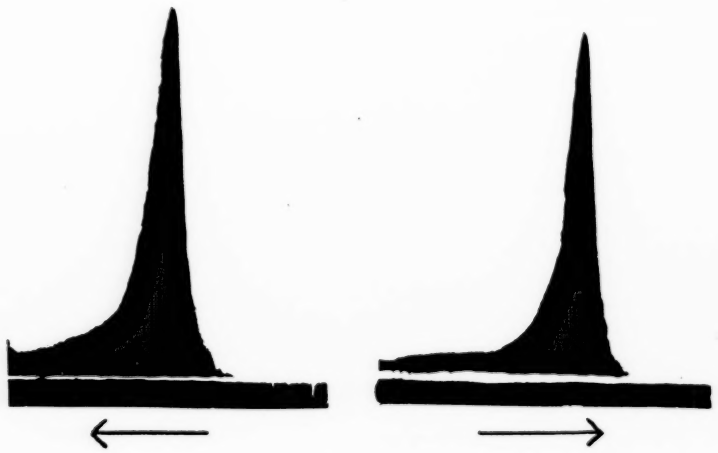

FIG. 2

FIG. 1.—Ascending pattern of preparation B24D in 0.02 M phosphate buffer (pH 7.4) + 0.15 M NaCl after 60 minutes.
FIG. 2.—Preparation C6D in 0.02 M phosphate buffer (pH 7.4). Left, descending pattern after 30 minutes; right, ascending pattern after 30 minutes.

SUMMARY

On high speed centrifugation of a saline solution of the material precipitated from a chicken sarcoma I extract by the addition of papain, a highly active fraction was obtained which was free of papain and electrophoretically homogeneous at pH 7.4.

The authors wish to express their appreciation to Dr. D. H. Moore for his aid in the electrophoresis experiments.

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Some Cytologic Effects of Repeated Doses of Radiation on Mouse Sarcoma 180

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The cumulative effect of repeated doses of radiation has been frequently reported. The literature has been reviewed by Duggar (2), Packard (4), Crowther (1), and others. The general conclusions are concerned with mitosis with no attention to centrioles. The effect of repeated doses of radiation on centrioles in mouse sarcoma 180 has never been reported. In a series of observations (3) on radiated rat carcinoma Walker 256, we noted that repetition of a dose of 2,400 r produced a decrease in the total number of induced gross abnormalities in mitotic figures and an increase in cells having more than the normal number of 2 centrioles. An experiment was set up to test the effect of several repeated doses of radiation on the frequency of the appearance of abnormal mitoses and multicentriolar cells at stated intervals after radiation.

METHOD OF PROCEDURE

Inbred strains of C57 black mice carrying sarcoma 180 were radiated with a dose of 1,200 r each at intervals of 10 days.[1] The type and dosage of radiation were as follows: 140,000 volt x-ray, 8 ma., 32 cm. distance. Filter ¼ Cu, 2 mm. Al, 3 mm. celluloid. 31.8 r.p.m. as measured in air. HBL 0.52 mm. Cu. Effective wave length 0.22. Dose 1,200 r. After 3 radiations the tumor was transplanted into the same strain of mice and the same procedure was followed for a 4th and 5th radiation. The tumor was transplanted into the flank of the animal so that when radiated practically all of the animal could be shielded with lead except the tumor. Even so by 35 days after the first radiation a histological examination of the testes of the mice that had been radiated 3 times showed a loss of nearly all the germinal cells. At intervals of 18, 24, 48, 72, 96, and 120 hours after each radiation, tissue was prepared for cytological study and comparison with unradiated tissue.

The cytological procedure was to observe the mitotic figures and the centrioles. For each preparation a total of 1,000 resting cells with identifiable centrioles were observed and their morphological characteristics noted. The centrioles occurring in each were counted. In other series of 1,000 viable cells each, mitotic figures were tabulated as normal or showing some atypical configuration such as polyploidy, various types of multipolar spindles, or fragmented or widely scattered chromosomes.

Fixation was in Zenker or Bouin solution. Eosin Y-methylene blue, aqueous alum hematoxylin, or Heidenhain's iron hematoxylin stain was used.

The growth rate of the radiated tumor was noted macroscopically and compared with the control non-radiated tumor.

OBSERVATIONS

In this experiment there were no regressions or any extended retardation of the growth of the tumor with 1,200 r. Immediately after each radiation there was a temporary retardation in growth, noted in the gross, but once increase in size was resumed it continued at a rate at least as great as in the controls.

Mitoses.—The effect of repeated doses of 1,200 r on the frequency of appearance of abnormal mitoses can be seen in Table I. Most occurred in the period from 24 to 48 hours after the first radiation. By 120 hours the abnormal mitoses were no more numerous than in the control. As a result of the second radiation the maximum effect was less, appeared later, and lasted longer. This is in agreement with previous findings (2) on the rat carcinoma Walker 256. After the 3rd radiation there was no appreciable increase in the number of abnormal mitoses over that found in the control. A radiation of 2,400 r on rat carcinoma Walker 256 caused giant cells, multipolar mitoses, mass accumulations of chromosomal substance, and other evidences of gigantism. With 1,200 r on mouse sarcoma 180 there was almost a complete absence of giant cells and of large, complex multipolar mitoses.

Throughout this study most of the abnormalities recorded were an apparent increase in the chromosomal substance, enlargement of the spindle, or fragmentation of chromosomes. For each 1,000 cells

[1] The tumor and mice necessary for the first part of this experiment were furnished through the courtesy of Dr. C. F. Branch, Boston University Medical School.

counted at any interval after the first or later radiations multipolar spindles were noted rarely.

Effect of transplantation.—The radiated cells were not affected in their ability to grow in a new host. The transplant tissue was taken from a 35-day large, necrotic tumor. There were good pearly-white peripheral foci of growth, however, from which the transplant was made. Among 25 animals inoculated there were 20 takes. By 10 days, 10 of these were of usual size and 10 somewhat smaller. The smaller ones subsequently grew actively. Fourteen tumors were radiated on the 12th day (4th radiation). Table I shows that the percentage of abnormal mitoses was not increased. Neither was there any indication that centrioles after the fixatives and stains used, as compared to Walker 256. When stained, however, they are definite entities, 2 in number for a normal unradiated cell. It has been suggested that when more than 2 centrioles appear there has been a suppression of cleavage but not of the function of the kinetic apparatus (3). In unradiated stock there is always a small percentage of the cells that have more than 2 centrioles. High doses (2,400 r) increase this number. Table II shows that the first dose of 1,200 r did not produce many more cells with over 2 centrioles than were present in the unradiated control. In those cells which had more than 2 centrioles 4 was the number most frequently found. We assume that there had

TABLE I: EFFECT OF REPEAT DOSES OF 1,200 r ON MOUSE SARCOMA 180

PERCENTAGE OF ABNORMAL MITOSES PER 1,000 FIGURES

Hours after radiation	First	Second	Third		Fourth	Fifth
18	6.7	—	—	TUMOR	—	—
24	17.6	4.4	4.7	TRANS-	5.0	3.3
48	14.2	9.2	5.1	PLANTED	3.2	3.1
72	5.0	9.3	3.8		3.7	3.1
96	5.2	5.9	4.2		4.7	4.6
120	4.4	4.9	—		3.2	4.2

Unradiated control .....4.6.
Unradiated after transplant.....4.4.

TABLE II: EFFECT OF REPEAT DOSES OF 1,200 r ON MOUSE SARCOMA 180

PERCENTAGE OF MULTICENTRIOLAR CELLS PER 1,000 CELLS IDENTIFIED WITH CENTRIOLES

Hours after radiation	First	Second	Third		Fourth	Fifth
24	—	5.3	6.1	TUMOR	—	—
48	5.4	5.2	7.5	TRANS-	8.3	—
72	5.0	5.0	7.0	PLANTED	9.5	10.7
96	5.5	9.3	7.6		8.8	11.4
120	5.5	6.9	9.9		9.8	13.0

Unradiated control .....3.0.

the 4th and 5th radiations had in any way retarded the rate of growth. Histologically there was no evidence of inhibition of growth. For each of the intervals studied abundant mitotic activity and absence of abnormalities persisted. For example, in 27 days after transplantation (from a small 10-day tumor) the last surviving mouse had such a large tumor that it was dying. This compares to 35 days after the first treatment or transplant.

A new host did not alter the trend of the effect of repeated radiation in respect to the frequency of mitoses abnormal in the gross. The trend is toward normal dividing cells in increasing numbers.

Centrioles.—The effect of repeated doses of 1,200 r on the percentage of cells containing more than 2 centrioles is shown in Table II. Mouse sarcoma 180 is not a favorable tissue for the demonstration of been a suppression of cleavage but that there was sufficient vitality for other mitotic processes to continue. This results either in 2 nuclei within a cell wall or a single large nucleus. If the environment is such that cleavage continues to be suppressed more than one cell generation, yet there is sufficient vitality for the mitotic processes to continue, we could expect to find in the interkinetic stage an increase in the number of centrioles. The more cell generations that occur under such a condition the higher the number of centrioles. If the increase were normal, centrioles would increase from 2 to 4, 4 to 8, 8 to 16, etc., but actually after the first duplication there is rarely a subsequent normal duplication. Our experience is to find 6, 8, 10, or 12 centrioles. Table III summarizes the data on multicentriolar cells. It can be seen from this table that after the first radiation there was a

total of 214 multicentriolar cells of the 4,000 observed. Of these 214 cells 189 had 4 centrioles. Therefore 25 cells of the 214 (11.7 per cent) had more than 4 centrioles. After the third radiation there were 481 multicentriolar cells of 5,000 counted. This is a slight increase in percentage over that found in the first radiation. The proportion of more than four multicentriolar cells to those with 4 was 53 to 328, or 14 per cent. On the same basis of calculation, after the 4th radiation the percentage was 17.8 and after the 5th, 21.9. Thus there is an increase in the number of cells showing the suppression of cleavage and an increase also in the number of cells which have not alter the trend toward an increase in the number of multicentriolar cells.

SUMMARY AND CONCLUSIONS

A quantitative study was made of the cumulative effect of repeated doses of 1,200 r at 10-day intervals on mouse sarcoma 180. After the 3rd radiation the tumor was transplanted to a new host of the same strain.

For the first 3 radiations there was only a temporary macroscopic retardation of growth immediately after each radiation. After transplantation it would appear

TABLE III: EFFECT OF REPEAT DOSES OF 1,200 r ON MOUSE SARCOMA 180

SUMMARY OF DATA ON MULTICENTRIOLAR CELLS

	First	Second	Third		Fourth	Fifth
Total cells counted	4,000	5,000	5,000	TUMOR	4,000	3,000
Number of multicentriolar cells	214	316	481	TRANS-	363	351
Percentage multicentriolar	5.4	6.3	7.6	PLANTED	9.0	11.7
Number of cells with 4 centrioles	189	273	328		298	274
Number of cells with more than 4 centrioles	25	43	53		65	77
Percentage of more than 4 centrioles to less than 4	11.7	13.6	14.0		17.8	21.9

TABLE IV: EFFECT OF REPEAT DOSES OF 1,200 r ON MOUSE SARCOMA 180

NUMBER OF MULTICENTRIOLAR CELLS 120 HOURS AFTER RADIATION

Dose	Number centrioles per cell						Total cells counted
	2	4	6	8	10	12	
Control	970	30	0	0	0	0	1,000
First	945	46	5	3	1	0	1,000
Second	931	60	6	1	0	1	1,000
Third	901	87	3	8	1	0	1,000
TUMOR TRANSPLANTED							
Fourth	902	82	9	6	1	0	1,000
Fifth	870	103	11	12	1	3	1,000

vitality enough to perpetuate divisions of cell components beyond the first division.

Table IV illustrates the same trend but shows the actual count for 1,000 cells with visible centrioles based on the number of centrioles per cell. The count was made from tissue prepared at the 120-hour interval after each of the 5 radiations and represents a breakdown of the total percentage of multicentriolar cells as listed for 120 hours in Table II. From this table it can be seen that the number of cells having 4 centrioles increases after each radiation. Also after each successive radiation there is a progressive increase in the number of cells having more than 4 centrioles. Intervals other than 120 hours would show this to a less degree. The interpretation of this awaits further investigation.

Effect of transplantation.—As in the case of abnormal mitoses the introduction of a new host did that the tumors were growing at least as fast as the unradiated control, based on observations covering 8 months and still being carried on.

The dosage (1,200 r) was sufficient to produce abnormal mitoses in a small percentage of the cells. The highest percentage occurs within the period of 24 to 48 hours after the first radiation. After the 3rd radiation there was no appreciable increase in the percentage of abnormal mitoses over that noted in the control.

The five doses of 1,200 r produced a gradual increase in the percentage of cells revealing more than 2 centrioles. This suggests that one effect of radiation is the prolonged suppression of cleavage without cessation of the function of the kinetic apparatus. The process continues through several abortive divisions, producing cells with 4, 6, 8, 10, or 12 centrioles. With this dosage no higher numbers were observed.

Some of the abnormalities of mitosis are of such a nature that they can produce cells with more than 4 centrioles. The presence of such cells in the tumor in increasing numbers is one way in which the increasing percentage in multicentriolar cells can be explained. Thus while the percentage of abnormal mitoses is more or less constant there is apparently an increase in the number of cells with the capacity to continue through several abortive divisions. This would account for the rise in percentage of multicentriolar cells.

It is recognized that this is not necessarily a specific effect of radiation.

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Abstracts

Reports of Experimental Research

Carcinogenic Compounds

BECK, S. [Research Dept., Glasgow Roy. Cancer Hosp., Glasgow, Scotland] SARCOMA PRODUCED BY SUBCUTANEOUS INJECTIONS OF OVERHEATED COTTON-SEED OIL INTO MICE. Brit. J. Exper. Path., 22:299-302. 1941.

Data from other workers indicate that (a) sarcoma can be induced in a small proportion of rats injected with lard alone, (b) mice on the other hand are not susceptible, and (c) heated domestic fats (when fed to rats) can induce stomach tumors.

Cottonseed oil heated to 350° C. for 1 hour was injected (0.5 cc.) subcutaneously into 12 mice; 6 survived more than 414 days when sarcoma appeared in one animal and in another on the 538th day. No tumors were found in 3 survivors living more than 414 days after the injection in 12 mice of oil heated for 12 hours at 210° C. Controls, 10 mice living more than 414 days after injection of unheated oil, were also negative.

Spectroscopic examination of the heated oils gave no indication of the presence of carcinogenic hydrocarbons. The long latent period in these tests suggests that either (a) absorption of the active factor is very slow or (b) that it is present in very low concentration.—I. H.

CHALMERS, J. G., and CROWFOOT, D. [Glasgow Royal Cancer Hosp., Scotland, and Dept. of Mineralogy and Crystallography, Oxford, England] THE ELIMINATION OF 3:4-BENZPYRENE FROM THE ANIMAL BODY AFTER SUBCUTANEOUS INJECTION. 2. CHANGED BENZPYRENE. Biochem. J., 35:1270-1275. 1941.

After subcutaneous injection into rats, 3,4-benzpyrene is in part excreted as a fluorescent derivative, BPX, in bile, feces, and urine. Purification by extraction, alumina adsorption, and vacuum sublimation gave a crystalline product. Solubility in alkalies suggests a hydroxy derivative, but melting point, fluorescence spectra, alkali solubility differences, morphological and optical examination of the crystals, and seeded recrystallization (hot wire stage), indicate that BPX is not identical with 6-hydroxy-3,4-benzpyrene or with 4′-hydroxy-3,4-benzpyrene. X-ray crystallographic examination and density measurements suggest that the BPX molecule has the dimensions required for a monohydroxybenzpyrene and exclude a dihydroxybenzpyrene.—I. H.

CHALMERS, J. G., and PEACOCK, P. R. [Research Dept. of Glasgow Royal Cancer Hosp., Scotland] THE EXCRETION OF DERIVATIVES OF CERTAIN CARCINOGENIC AND NON-CARCINOGENIC HYDROCARBONS IN FOWL BILE. Biochem. J., 35:1276-1282. 1941.

Five carcinogenic hydrocarbons, (1) 3,4-benzphenanthrene, (2) 3,4-benzpyrene, (3) 1,2,5,6-dibenzanthracene, (4) cholanthrene, (5) methylcholanthrene, and six non-carcinogenic hydrocarbons, (6) anthracene, (7) phenanthrene, (8) pyrene, (9) 2′, 6-dimethyl-1,2-benzanthracene, (10) 2′,7-dimethyl-1,2-benzanthracene, (11) fluoranthene, were injected separately into fowls (about 2.0 mgm. of colloidal solution, intravenously). Bile from these fowls obtained *via* cholecystostomy fistulae was extracted with ether and examined by fluorescence spectroscopy for hydrocarbons after alumina adsorption purification. The NaOH washings of the ether extracts were examined for derivatives of the hydrocarbons.

Of the eleven hydrocarbons only (2) could be detected in the bile, but the limits of sensitivity of the technic in general have not yet been satisfactorily determined.

Examination of the NaOH washings for hydrocarbon derivatives indicated that (2), (BPX, see preceding abstract), (8), and (9) gave rise to products showing banded fluorescence spectra; (3), (4), (5), (6), (10), and (11) gave products with general fluorescence; and (1) and (7) gave nonfluorescent products.—I. H.

STEINER, P. E. [Dept. of Pathology, Univ. of Chicago, Chicago, Ill.] THE INDUCTION OF TUMORS WITH EXTRACTS FROM HUMAN LIVERS AND HUMAN CANCERS. Cancer Research, 2:425-435. 1942.

Twelve sarcomas were induced in 37 mice at the site of subcutaneous injection of the nonsaponifiable lipid fraction extracted from pooled noncancerous livers of persons who died with cancer. The induction time was 182 days and the percentage yield was 32.4.

An extract similarly prepared from the livers of non-cancer-bearing persons had less carcinogenic activity, having an induction time of 12 months and a percentage yield of 14.3 (5 sarcomas in 35 mice). Neither of these extracts induced tumors in rats.

A benzene extract of liver from a person who died with cancer, and various fractions of such an extract failed to induce tumors at the site of injection.

Extracts of cancer tissues also did not induce tumors.

A theory for the chemical causation of cancer is outlined, and its possible relationship to the theory of chronic irritation is pointed out.—Author's abstract.

SYVERTON, J. T., BERRY, G. P., and DASCOMB, H. E. [Univ. of Rochester, Rochester, N. Y.] STUDIES ON CARCINOGENESIS IN RABBITS. I. MALIGNANT TUMORS INDUCED IN COTTONTAIL RABBITS BY THE INJECTION OF METHYLCHOLANTHRENE IN TRICAPRYLIN. Cancer Research, 2:436-444. 1942.

Methylcholanthrene was used to produce malignant tumors in the cottontail rabbit, a new host for this type of work. The carcinogenic agent was injected into each of 11 cottontails at 4 sites, 2 subcutaneous and 2 intramuscular, in amounts of 250 mgm. in 1 ml. of tricaprylin.

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These animals were permitted to survive without operative interference for the duration of their natural lives. Five died before the 175th day from intercurrent infection or injury; at the time of death no histological evidence of neoplasia was found. The remaining 6 died between the 176th and the 295th day after injection. Of this group, 2 had carcinomas, and 5 had soft-tissue sarcomas with metastases to a regional lymph node in 2 and in a single animal to the lungs, liver, and kidneys in addition. Attempts to transmit the methylcholanthrene-induced tumors by serial passage in cottontail rabbits were unsuccessful. No evidence was encountered to suggest that a virus was playing any rôle in the reactions observed—Authors' abstract.

WASLEY, W. L., and RUSCH, H. P. [Univ. of Wisconsin, Madison, Wis.] INHIBITION OF THE AUTOXIDATION OF ALDEHYDES BY CARCINOGENIC CHEMICALS AND RELATED COMPOUNDS. Cancer Research, 2:422-424. 1942.

The autoxidation of benzaldehyde and of heptaldehyde in the presence of anthracene, 3,4-benzpyrene, 1,2,5,6-dibenzanthracene, 20-methylcholanthrene, phenanthrene, dimethylaminoazobenzene, and hydroquinone in concentrations of M/1,000 to M/10,000 was studied in an ordinary Warburg manometric apparatus at 24° C. All these compounds except phenanthrene noticeably inhibited the autoxidation of the aldehydes. Evidence from absorption spectra indicated that the inhibition of the autoxidation of benzaldehyde by 3,4-benzpyrene involves the induced oxidation of the latter to one or more quinones.—Authors' abstract.

WOOD, J. L., and FIESER, L. F. [Harvard Univ., Cambridge, Mass.] THIOCYANATION OF CARCINOGENIC HYDROCARBONS. J. Am. Chem. Soc., 63:2323-2331. 1941.

Certain sulfhydryl and cysteine derivatives of 1,2-benzanthracene, 10-methyl-1,2-benzanthracene and 3,4-benzpyrene (J. Am. Chem. Soc., **62**:2674. 1940) have been found not to exhibit any carcinogenic activity. It is concluded that these sulfur-substituted derivatives cannot function as intermediates in the process of hydrocarbon carcinogenesis but that these or isomeric substances may possibly afford one route for the metabolic detoxification of the hydrocarbons. The observations on the thiocyanation of carcinogenic hydrocarbons support the view that the initiation of carcinogenesis by a hydrocarbon is associated with a substitution into either a meso position or an alkyl group located at such a position and involves interaction with a cell constituent containing a disulfide group. The opening of a disulfide linkage of a protein might occur by the utilization of either an active nuclear hydrogen atom or the hydrogen of a meso methyl or methylene group.—H. J. C.

Biochemistry and Nutrition

COHEN, P. P., HEKUIS, G. L., and SOBER, E. K. [Yale Univ. Sch. of Med., New Haven, Conn., and Univ. of Wisconsin, Madison, Wis.] TRANSAMINATION IN LIVER FROM RATS FED BUTTER YELLOW. Cancer Research, 2:405-410. 1942.

The transaminase activity of livers from rats fed a brown rice diet, with and without butter yellow, was determined at various intervals up to 200 days. The changes in these livers were followed by histological examination. With continued butter yellow feeding it was observed that the transaminase activity fell to one-third the initial value in the resulting tumor tissue. There was a high correlation between the transaminase activity and the days of butter yellow feeding. Dilution experiments resulted in curves which showed progressively decreasing values for transaminase activity. The dilution curves for the butter yellow tumor tissue were practically identical with those previously reported for transplanted mouse tumors. Livers from rats fed 15% yeast in addition to the rice-butter yellow diet showed normal values. The inhibitory effect on transaminase of the following metabolic intermediates of butter yellow, arranged in order of their potency, was investigated: quinone, N-methyl-*p*-phenylenediamine, N,N-dimethyl-*p*-phenylenediamine, and *p*-phenylenediamine.—Authors' abstract.

Leukemia

HALL, V. E., and FURTH, J. [Cornell Univ. Med. Coll., New York, N. Y.] METABOLIC STUDIES IN MOUSE LEUKEMIA. I. THE METABOLISM OF LYMPH NODES IN LYMPHOID LEUKEMIA. Cancer Research, 2:411-421. 1942.

The oxygen consumption of the lymph nodes of mice with spontaneous and transmitted lymphoid leukemia is not significantly different from that of normal lymph nodes. The rate of aerobic glycolysis is often, but not invariably, increased; that of anaerobic glycolysis is invariably increased. The Pasteur effect is greatly increased in the leukemic as compared with the normal lymphoid tissues studied. The metabolic activity of the leukemic tissue is similar to that of malignant tumors of mice but differs from that of human and rat tumors by a lower rate of aerobic glycolysis and a slightly negative value for the fermentation excess.

In leukemic hybrids between high and low leukemia stock mice, the rate of oxygen consumption is higher and that of aerobic and anaerobic glycolysis is lower than in high leukemia stock mice. The relative significance of the percentage inheritance from leukemia-resistant and leukemia-susceptible stock remains to be determined.

In those leukemic lymph nodes which contain considerable normal tissue, the aerobic glycolysis rate of the malignant lymphocytes is higher and the anaerobic glycolysis rate is lower than in the nodes consisting almost entirely of malignant cells. With increasing age the character of the metabolism of the leukemic lymph nodes changes, the aerobic glycolysis rate increasing, the anaerobic glycolysis rate decreasing. These changes appear to be due rather to differences in the glycolytic activities of the lymph nodes themselves than to factors in the body of the host.—Authors' summary.

Transplantation

CASEY, A. E., PEARCE, L., and ROSAHN, P. D. [Rockefeller Inst. for Med. Research, New York, N. Y., Sch. of Med., St. Louis Univ., St. Louis, Mo., and Sch. of Med., Louisiana State Univ., New Orleans, La.] BLOOD CELL FACTORS IN THE METASTASIS OF THE BROWN-PEARCE TUMOR. Cancer Research, 2:401-404. 1942.

One hundred and eight rabbits apparently free from intercurrent disease and having each of 9 blood factor

levels within normal limits were inoculated intratesticularly with the Brown-Pearce tumor. In 90 the grafts were successful. Among these, high eosinophile and low blood platelet pretransplantation levels were associated each with fewer metastases and a lower mortality than was the case with low eosinophile and high blood platelet values. Intermediate values of the red blood cell and basophile pretransplantation levels were associated with fewer metastases and a lower mortality from the neoplasm than were extreme values. No significant correlation existed between the pretransplantation blood levels of the hemoglobin, the total white blood cells, the neutrophiles, the lymphocytes, or of the monocytes, and: (1) the incidence of metastases, (2) the mortality from the neoplasm, or (3) the number of metastatic foci developed. The host factors which influenced the success of transplantation were different from those which influenced its continued growth and spread and the mortality from the neoplasm.—Authors' abstract.

Treatment—Radiation, Chemotherapy, etc.

BOYLAND, E. [Chester Beatty Research Inst., Royal Cancer Hosp. (Free), London] EXPERIMENTS ON THE CHEMOTHERAPY OF CANCER. 5. THE EFFECT OF MUSCLE EXTRACT AND ALIPHATIC BASES. Biochem. J., 35:1283-1288. 1941.

Previous work (Roffo) has shown that muscle extracts inhibit tumor growth. Muscle was acid-extracted, dialyzed, precipitated with trichloracetic acid, and the filtrate precipitated with phosphotungstic acid for the preparation of the bases. At each stage tests (daily oral administration; about 1/5th the lethal dose) were made on spontaneous mouse carcinoma and on grafted mouse sarcoma M.C.D.B.I., using the muscle fractions, the bases likely to occur, and related compounds (β-alanine, aneurin, arcaine sulfate, betaine, carnosine nitrate, choline chloride, creatine, creatinine, ethanolamine, methylguanidine, trimethylamine oxide hydrochloride, ethylenediamine hydrochloride, putrescine, cadaverine hydrochloride, spermine hydrochloride, tetradecamethylenediamine dihydrochloride).

Ethanolamine and cadaverine hydrochloride were the most effective inhibitors but were not much superior to the "muscle extract trichloracetic acid-soluble fraction." These results (a) indicate that the activity of the muscle fraction is due to a combination of factors and (b) suggest some speculations on the correlation of chemical structure and tumor growth-inhibiting capacity.—I. H.

Clinical and Pathological Reports

Breast

CHILKO, A. J., and QUASTLER, H. [New Rochelle Hosp., New Rochelle, N. Y.] DELAYED METASTASES IN CANCER OF THE BREAST. Am. J. Surg., 55:75-82. 1942.

An analysis of 29 cases of recurrence after apparently successful operation has been made, but no evidence of qualitative difference between cases with long and short latency was found, except that latency was shorter in cases with rapid preoperative growth or with local metastases at the time of operation. On comparison of cases of breast cancer with delayed metastases compiled from the literature no trait common to all these cases was found.—H. G. W.

LOGIE, J. W. [Univ. of Michigan, Ann Arbor, Mich.] MASTOPATHIA CYSTICA AND MAMMARY CARCINOMA. Cancer Research, 2:394-397. 1942.

The relationship of cystic disease of the breast to mammary carcinoma has long been a subject of controversy, yet practical observation frequently reveals the concomitance of the two conditions. The criteria for the recognition of mastopathia cystica, as applied to the present study, included dilatation of ducts with accompanying fibrosis; areas of "pale-celled" hypertrophy of ductal epithelium, of the type found in the apocrine sweat glands; papillary ingrowths, to varying degree, into cystic ducts, and, frequently, hyperplasia of terminal ducts and acini. Operative material from the breasts of 330 women was re-examined. One hundred and eighteen specimens were carcinomatous, and of these 67 showed coexisting mastopathia cystica. Of 212 breasts without carcinoma, 82 showed mastopathia cystica. Application of the χ^2 test to these data indicated that the chance of obtaining such a degree of concomitance, if mastopathia cystica and mammary carcinoma are independent, is less than 1 in 1,000. In practical diagnostic experience, carcinoma is frequently found arising in areas of mastopathia cystica. This was true of 13.5% of the cancers of the breast included in this analysis. Upon both statistical and histopathologic grounds a causal relationship between mastopathia cystica and mammary carcinoma must be accepted.—Author's abstract.

MACDONALD, J. [Los Angeles, Calif.] MAMMARY CARCINOMA. Surg., Gynec. & Obst., 74:75-82. 1942.

The records of cured cancer cases of the American College of Surgeons contain in 2,636 cases the records of 1,511 5-year cures and 1,125 cases in which recurrences or metastases developed within the 5-year period. The age of the patients does not have as much prognostic significance as ordinarily supposed, and cancer of the breast at an early age warrants radical approach with as much chance of cure as in older women. Relatives of women with breast cancer have an incidence of breast cancer 3 times greater than the general population. Nulliparae are more prone to develop cancer of the breast than women who have borne children, but the prognosis is as good or better with the nulliparae. Tumors of long duration are no contraindication to radical treatment, for 25% of the 5-year cures were treated more than 1 year after recognition of the tumor.—H. G. W.

MELAND, O. N. [Los Angeles Tumor Inst., Los Angeles, Calif.] THE INFLUENCE OF RADIATION ON LONGEVITY IN CANCER OF THE BREAST. J. A. M. A., 118:274-277. 1942.

This paper based on a study of 803 followed up cases of cancer of the breast treated by radiation with or without surgery, leads to the conclusion that radiation plays a dominant role in the treatment. It contributes to longevity in all groups with the exception of group 1 in which radical removal alone is apparently sufficient. In group 2, preoperative irradiation as an adjunct to surgery increases the rate

of survival between 15 and 20%. While irradiation alone does not give the results that surgery does, interstitial irradiation approaches it.—H. G. W.

SACHS, M. D. [Univ. of Oregon Med. School, Portland, Oreg.] CARCINOMA OF THE MALE BREAST. Radiology, 37:458-467. 1941.

Two hundred and five cases of carcinoma of the male breast were collected by means of questionnaires sent to leading radiologists, surgeons, and pathologists in the United States and Canada. These are analyzed together with 436 cases from the literature. Male breast carcinoma makes up about 0.7% of all male carcinoma and bears a ratio of 1.16% to female breast cancer. Tables are presented, showing the age incidence, duration of symptoms, type of tumor, location of metastases, and end results. The average age of the patients was 57 years. The tumors were predominantly adenocarcinomas. Local recurrence was noted in 25% of the cases and metastases were diagnosed clinically in 46%. The prognosis of carcinoma of the male breast is considered poor. Most of the patients received adequate treatment but only 7.5% were living and well at the end of 5 years.—C. E. D.

STEIN, R. J. [Orleans County Memorial Hosp., Newport, Vt.] FIBROLEIOMYOMA OF THE BREAST. Arch. Path., 33:72-74. 1942.

A case of primary parenchymal fibromyoma of the breast is added to the 4 hitherto reported.—H. G. W.

Male Genital Tract

HERGER, C. C., and SAUER, H. R. [State Inst. for Study of Malignant Diseases, Buffalo, N. Y.] FURTHER OBSERVATION ON SERUM ACID PHOSPHATASE ACTIVITY IN CARCINOMA OF THE PROSTATE. Cancer Research, 2:398-400. 1942.

Data on serum acid phosphatase determination collected over a period of 2 years are presented.

Determinations were made on 430 patients, 147 of whom had carcinoma of the prostate, while 283 were control cases. These studies show that serum acid phosphatase levels up to 4.0 King-Armstrong units should be considered normal. Values of from 4.0 to 6.0 units represent borderline figures, which have no diagnostic value so long as no further increase of the acid phosphatase occurs subsequently. A definite rise indicates metastatic bone involvement from carcinoma of the prostate. A distinct elevation was found in the majority of patients in whom metastatic bone lesions were demonstrated roentgenologically.

Levels of more than 100 King-Armstrong units are to be regarded as an unfavorable prognostic sign.

If elevation of the serum acid phosphatase develops, there is a tendency to steady increase providing no effective treatment is initiated.—Authors' abstract.

Oral Cavity and Upper Respiratory Tract

ABELS, J. C., REKERS, P. E., MARTIN, H., and RHOADS, C. P. [Memorial Hosp., New York, N. Y.] THE RELATIONSHIP BETWEEN DIETARY DEFICIENCY AND THE OCCURRENCE OF PAPILLARY ATROPHY OF THE TONGUE AND ORAL LEUKOPLAKIA. Cancer Research, 2:381-393. 1942.

Causative factors for atrophy of glossal papillae or oral leukoplakia were believed to be dietary factors and excessive use of tobacco rather than syphilis.

Pathological changes most frequently associated with glossal papillary atrophy and oral leukoplakia were gastric achlorhydria, functional and organic changes in the gastrointestinal tract, cheiloses and perleche, onychia, and occasionally varying degrees and types of anemia.

To test the relationship between the inadequate ingestion of protein and vitamin B complex, and the occurrence of papillary atrophy of the tongue and oral leukoplakia, a crude granular yeast preparation was administered orally. This treatment was followed occasionally by a complete, and frequently by a partial, remission of the oral lesion and oral symptoms.—Authors' abstract.

Miscellaneous

WILLIS, R. A. [Depts. of Pathology of the Alfred Hosp., Prahran, and of the Univ. of Melbourne, Melbourne, Australia] A REVIEW OF FIVE HUNDRED CONSECUTIVE CANCER AUTOPSIES. Med. J. Australia, 2:258-265. 1941.

This paper is difficult to abstract and should be read in the original. It supplements Willis' treatise on *The Spread of Tumours in the Human Body,* J. and A. Churchill, London, 1934, by bringing up to 500 the 323 consecutive autopsies on subjects of malignant disease there described; and its object is to summarize the findings in the completed series. Following a short description of the autopsy technic employed, the paper takes the form of a review on a regional and statistical basis, with special reference to modes of extension and the situations of metastases. More detailed accounts are given of various noteworthy items, and attention may be directed to one (case 332) because of the youth of the patient and remarkable variations in the rate of growth of the tumor (massive carcinoma simplex of the breast showing extraordinary mitotic activity) during and between pregnancies. Features of this case were great initial activity of the growth during lactation, a subsequent 12 months' quiescence in spite of incomplete removal, and sudden reappearance and rapid growth in the opposite breast during the latter part of later pregnancy and lactation. "These facts suggest that hormonal influences play a part, not only in the genesis of mammary cancer, but also in its rate of growth."—A. H.